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CONTENTS

The Catalytic Element in Science

Little by little, science pulls away the shrouds of secrecy that surround the physical forces of the universe. Each new generation tries valiantly to contribute its share of new knowledge. Sometimes the advancements are substantial; sometimes they fall short. The difference lies in the stature of the scholars which each generation is capable of producing. Fortunately, most disciplines have been blessed with a genius or two whose ideas and discoveries continue to stimulate and to teach vicariously, thus serving to open new doors. The lead article in this issue of *Review* illustrates this principle well. Without the opportunity to draw on the funds of knowledge established by Darwin, Mendel, Weismann, and other towering figures in the history of science, geneticist Wallace would have been unable to log his success with mutagenic agents.

Emerging at this point is the significant observation that these early scholars have unwittingly given us the means of utilizing fully the "breathing spell" that Wallace speaks of—that extra bonus of time for developing new breeding techniques before our natural genetic variability becomes exhausted. Therein lies one example of the catalytic element in science, which is kept alive, so to speak, and passed along from one generation to another.

Increment by increment, as man's repository of knowledge grows, the shackles of want and ignorance weaken. Perhaps it is not too much to expect that the growth of technology will enhance the reign of man in other equally desirable respects. At that point in the stream of history, Bacon's dream that man might partially recover his empire over creation will become a reality.

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Mutagenic Agents

Their Use for Plant and Animal Improvement

A. T. Wallace

TO produce new strains of plants and animals, nature continuously uses two notably successful processes—spontaneous mutations and natural hybridization, or combinations of the two. Historically, plant and animal breeders have concentrated their efforts on hybridization. Only in recent years has there been any major effort directed toward using the induced mutation process, even though the technique for inducing mutations by radiations has been known since 1927 (*14*).[1] It is the unpredictability and randomness of artificially induced mutations, coupled with their usually harmful effects, that no doubt account for their failure to be used to any extent by plant and animal breeders.

During the period immediately after Stadler's (*22*) report that he had been able to induce mutations in higher plants, there was considerable flurry by plant breeders to use this technique in their plant improvement programs. Because of the above-mentioned limitations, most plant breeders soon lost interest, and the technique was almost abandoned except by a small group in Sweden (*11*). On the heels of the atomic age after World War II, however, came its revival. Immediately after the war, many plant breeders began irradiating plants and plant parts. Here again, as expected, practical results were discouraging. A few new varieties were

[1] Italic numbers in parentheses refer to "Literature Cited" p. 8.

developed as the result of the use of mutagenic agents, but their number was significantly small.

The technique of artificially inducing mutations and using them to develop superior strains is called "mutation breeding," which is merely an additional tool for use in plant and animal improvement programs. It has great potentialities, of course, but a tremendous amount of research is necessary before mutation breeding can be fully utilized.

In recent years, the upsurge in mutation research has been so great that it has become an important part of modern genetics research. Unfortunately, from the plant and animal breeders' viewpoint, most of this research has been with microorganisms. Although it is very expensive to conduct mutation research with higher animals and plants, research with microorganisms is reaching the stage now that some of it should certainly be tested on the higher organisms. It is notable that some of the results from mutation research on microorganisms indicate that many of the desired mutations can be induced. If it became possible to induce and direct mutations in domestic plants and animals, the application of such results would be of immense value.

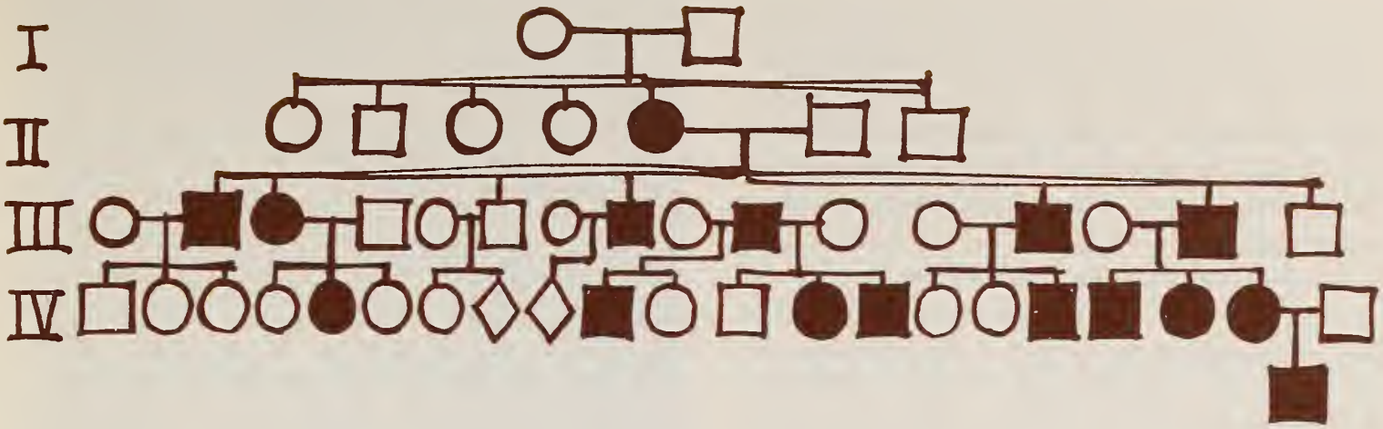
Because mutations are the source of all hereditary variability, evolution or plant and animal improvement, or both, would not be possible without them. When it is desirable to adapt crops to new environments beyond their normal range or when the natural gene variability is exhausted, the use of induced mutations offers real potentialities. One only has to let his imagination roam briefly to visualize the benefits of "adapting" crops to new environments. Furthermore, with certain crops, natural genetic variability is already near the point of exhaustion. This is especially true now in terms of disease resistance on some of the cereals. In addition, artificially induced mutations could be used to produce new or entirely different characteristics, now considered impossible, in plants and animals.

KINDS OF MUTATIONS

MUTATIONS occur spontaneously through an error in gene replication; or they may be *artificially* induced by a physical or chemical stimulus. For the sake of convenience, mutations are classified as intergenic changes or intragenic changes. The intergenic changes include all chromosomal changes (translocations, inversions, deletions, duplications) and those involving changes in chromosome numbers. Intragenic changes, or "true" gene mutations, include those that occur within the gene. However, because of the continuously changing concept of what a gene really is, it is difficult to pinpoint the difference between intra-and-intergenic changes. There is no clear dividing line between the two; one grades into the other.

Some controversy has developed among plant geneticists as to whether or not intragenic changes, *i.e.*, the so-called "true" gene mutations, can be induced in higher plants with ionizing radiations. The controversy may be illustrated by a statement by Rhoades (*17*) that there is no convincing evidence to support the theory of induced intragenic changes in higher plants. This sort of viewpoint has led a number of plant geneticists to believe that intragenic changes cannot be induced in higher plants with ionizing radiations. Other plant geneticists, recognizing the lack of such evidence, nevertheless believe that intragenic changes in higher plants not only *are* possible, but have, in fact, been induced with ionizing radiations. They believe that the relatively low rates of induced mutations, the difficulty of conducting specific loci studies with higher plants, and the number of different extragenic events that can stimulate gene mutation have prevented the accumulation of sufficient convincing evidence. They believe it is just a matter of time until such evidence is forthcoming with higher plants.

The above viewpoints are presented only to indicate that a number of plant breeders do not feel that mutation breeding has many practical benefits to offer. It appears, however, that the plant geneticists who believe that "true" gene mutations can be induced in higher plants with ionizing radiations have the bulk of evidence on their side of the discussion, provided that it is permissible to extrapolate from results obtained from microorganisms to those of higher plants. This support comes from the fact that true gene mutations have already been induced in microorganisms and even in *Drosophila*. For example, Benzer (*3*) has determined the forward mutation rate at a particular site in bacteria phage and then obtained reverse mutants from these mutants, *i.e.*, mutants back to the original form. Then, using these reverse mutants, he determined the forward mutation rates and found that in 8 out of 9



cases the forward mutation rate was the same as the original forward rate. These experiments of forward, reverse, and forward again provide convincing evidence that true gene mutations can be induced. In the process of evolution, the genetic systems of higher plants have come to differ rather markedly from those of microorganisms. Nevertheless, there are some common denominators—for instance, the gene and its basic function in all cells, and the common ingredient, DNA. Along with other genetic evidence, these common characteristics justify the assumption that there are great similarities between genes of microorganisms and those of higher plants, and that it is possible to extrapolate to some degree from the data of microorganism to that of higher plants.

USE OF MUTATIONS

IN plant improvement programs, both intergenic and intragenic mutations are considered potentially beneficial. The specific type will, of course, depend on the particular crop under investigation, its method of reproduction, its degree of ploidy and the availability of other sources of genetic variability. MacKey (*13*) has cited the following uses to specify the merits of mutation breeding: (a) adding a single characteristic to a delicate system of genic balance in which recombination may cause a breakdown of the whole system; (b) adding specific characteristics to heterozygous clones which hardly allow a purposeful recombination breeding; (c) splitting up very close linkage or gene block and in transferring small segments from one nonhomologous chromosome to another; (d) diploidization of artificial polyploids in order to facilitate a differentiation of identical or two closely related chromosome pairs; (e) producing hereditary changes that allow another breeding technique to be applied to that certain plant; and (f) producing entirely new hereditary constituents.

MacKey's last point is the one which carries the most important potential. It is also the one around which the controversy (mentioned earlier) has developed. However, further discussion in this paper will be based on the assumption that intragenic changes *can* be induced with ionizing radiations in higher plants as in microorganisms.

MODIFICATION OF IONIZING RADIATION EFFECTS

IT is known that all ionizing radiations will produce mutation in all living organisms. By definition, ionizing radiations are those that produce ion pairs when they interact with matter. This ionization, as it is called, changes the molecular structure of the cell's vital hereditary mechanism. In addition to the process of ionization, energy transfer also occurs by a process known as excitation. Ultraviolet light, which does not have the ability to ionize but transfers its energy by excitation, will also produce mutation. Thus, we can deduce that the process of excitation is also a means by which ionizing radiations will produce mutations. Regardless of its origin, ionizing particles will produce mutations. However, external sources of radiation have proved to be more practical for mutagenic purposes than having the plants and animals absorb the radioisotopes internally.

Sparrow (*21*) states that one of the difficulties with the state of modern radiobiology of higher

plants is a plethora of facts and a deficiency of general principles. He supports his statements by presenting a long list of factors that will modify the radiation response of plants, including 11 different groups of physical factors, 16 different groups of chemical factors, 11 different physiological factors, and 15 different biological factors. This list is long and impressive. To some degree it is also discouraging, for in only a few cases is the exact mechanism of modification known. It is especially discouraging to plant and animal breeders who desire to conduct mutation experiments. In order to have reproducible results, all variables, of course, must be controlled. Yet on the other hand, the fact that there are so many modifying factors offers a possibility and a challenge to plant and animal breeders to utilize these variables for increasing the effectiveness of mutagenic agents, and even for the possibility of partially directing the mutagenic event. This last possibility—knowing how to direct mutations—would be an immensely valuable contribution to plant and animal breeding.

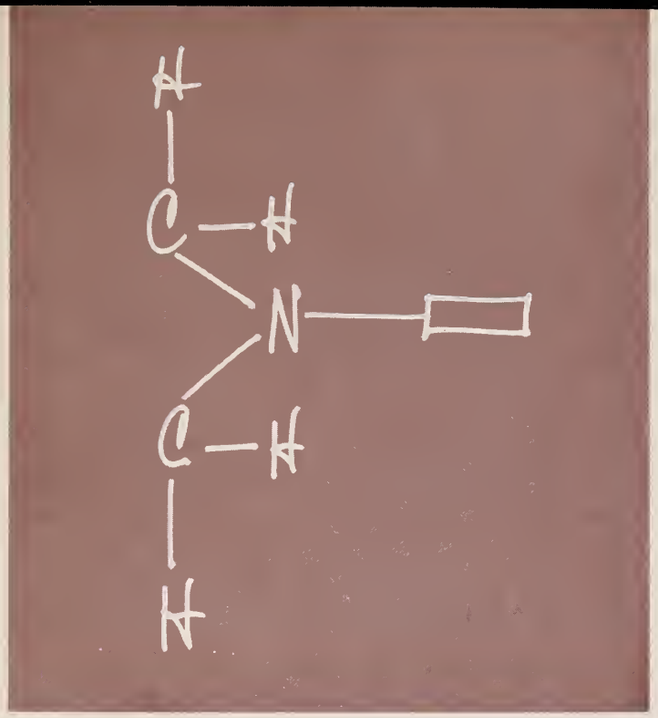
INCREASED MUTATION EFFICIENCY

A MAJOR problem that faces plant and animal breeders who are investigating mutation breeding techniques is the low rate of mutations that can be induced in a specific gene. This rate, of course, varies with the individual gene under investigation. However, most of the reports indicate that the average induced rate per locus under standard conditions usually ranges from 2 to 25×10^{-8} per roentgen. By the use of modifying agents, seeds of plants can be made to survive doses of irradiation ranging from 10,000 to 100,000 roentgens. With such doses the expected mutation frequency in any one gene can be as high as 2 to 25×10^{-3} per plant. This is a workable rate in most plant breeding programs.

A great deal of the mutant research in higher plants has been conducted with barley, in which the chromosome aberrations are determined in the M_1 generations and the frequency of chlorophyll deficiencies determined in the M_2. These are the first and second generations respectively following seed treatments with mutagenic agents. Using this procedure, Swedish workers (*7*) have compared the mutagenic effects of chemicals and ionizing radiations. Chemical mutagens generally produce the higher frequency of intragenic changes. Yet chemicals differ widely in their relative abilities to produce intergenic and intragenic changes (*2*). Two chemicals, both purines, may be cited to illustrate this point. With barley, 8-ethoxycaffeine produces almost entirely chromosome changes, whereas nebularine produces almost entirely gene mutations. Broken chromosome fragments produced by 8-ethoxycaffeine in *Vicia* readily form rearrangements while they remain as free fragments in *Allium*. Contrastingly, urethane produces mainly fragments in *Vicia,* but mainly rearrangements in *Oenothera.* By the proper selection of chemicals, one may predict that some day high mutation rates per offspring will be possible.

As an example of the use of modifying agents to increase the efficiency of ionizing radiations, the following may be cited. In these experiments, a series of Cobalt-60 gamma ray doses was given to oat seed at each of the following moisture percentage levels: 2.5, 3.5, 6.8, 10, and 90 by weight. The average mutation frequencies at a single locus, on a panicle basis, observed at these moisture levels were 0.5, 171.7, 14.8, 4.9, and 7.7 ($\times 10^{-8}$) per unit of dose, (roentgen), respectively (*24*). By merely varying the seed moisture content, one can vary the mutation efficiency of Cobalt-60 gamma rays over a 340-fold range.

These same data can be used to illustrate another point. That point relates to the controversy discussed earlier in this article. One of the reasons given to support the view that ionizing radiations cannot induce true gene mutations in higher plants is that of the high energy level usually associated with ionizations. In most cases, this energy level is probably sufficient to cause changes that are too drastic to be true gene mutations. The high rate of mutation at the 3.5 percent moisture level reported above (171.7×10^{-8}/r) is no doubt due, in a large measure, to indirect effects of the gamma rays. Since it would be expected that the energy level associated with the indirect effects would be, on the average, much lower than those associated with the direct effects, the expected change in the gene molecule should be less drastic. A proportion of these changes produced at this low moisture level are then assumed to be true gene mutations. This viewpoint is supported by the fact that ultraviolet light, which produces mutations by excitation (caused by lower energy levels than necessary for



ionizations) can apparently produce true gene mutations in higher plants. Because of its low penetrating ability, ultraviolet light has a very limited use for producing mutations in higher plants. By varying the seed moisture content, the same results can be obtained with ionizing radiations.

DIRECTED MUTATION—A POSSIBILITY?

AN ultimate objective of plant and animal breeders, of course, is to be able to induce a single mutation to the exclusion of all others. However, as Smith (*20*) suggests, a more legitimate objective—on the basis of the present working hypotheses about the structures that store and transmit genetic information—would be one that gives limited control of the spectra of mutations rather than a complete "all-or-none" direction of specific mutations. Already there is sufficient evidence that this not only can be, but is already being done.

As an example of this direction diethyl sulfate, a mutation-producing chemical, was combined with gamma rays and we obtained a mutation rate of 2.5 times that of the sum of the two agents when given separately.[2] Germination and seedling height tests indicated that this synergistic effect was not due to increased chromosome damage. Although no explanation for this synergism can be given, the following is known: The ethyl group of diethyl sulfate attaches itself to the DNA molecule, usually to the 7th nitrogen of guanine. A certain fraction of the ethylated guanine molecules breaks away from the DNA and this results in mutations (*9*). Apparently, having the ethyl group attached to guanine weakens its bonding to adjacent molecules. Thus, its weak bonds made it more susceptible to being changed by energy from the radiation. One may postulate, then, that in its weakened condition, the gene can be mutated at the guanine site by a low energy level that normally would have little or no effect. One could further postulate that the mutations would be concentrated at the gaunine site in the DNA. This could be thought of as mutation direction at the "base" level. Since, however, it would be expected that guanine is present in most, if not all, genes, this "base" level of mutation direction would be of limited value. With it, mutation direction at the "gene" level would be limited to those loci with a higher proportion of guanine.

In another experiment we obtained a synergistic effect of ethylene imine combined with gamma rays (*23*). It is thought that ethylene imine will alkylate the pyrimidines (*20*) and not the purines, as does diethyl sulfate. Thus, by changing the preirradiation treatment of seed from one chemical to another, we not only have changed the mutagenic efficiency of the ionizing radiation, but we can assume that we have changed the degree of specificity from purines to pyrimidines.

What we have described above is mutation direction at the "base" level. On the other hand, if there are specific proteins associated with specific loci, then one might postulate the possibility of mutation direction at the "gene" level. As a matter of fact, evidence shows that mutation direction can be obtained at the "gene" level. For example, many of the chemical mutagens produce more breaks in heterchromatic than in euchromatic regions. In *Drosophila,* the distribution of mutations over the sex chromosomes differs with the chemical. In phage, certain chemicals produce a higher frequency of mutations at preferred sites. In the silkworm, two linked genes determining the color of embryonic membranes appear to mutate in different ratios after treatment with X-rays and certain chemicals (*2*). In barley, the spectrum of chlorophyll mutations will vary with the mutagen used. Other examples may be cited, but these are sufficient to support the thesis that mutation direction is possible at the "gene" level. Admittedly, the direction is limited, but it is a promising step towards the ultimate goal.

[2] Wallace, A. T. 1964. Unpublished results.

It appears that the interaction between a mutagen and a locus is a semispecific process. The process can be influenced by many factors, including the conditions of the cell preceding and following the mutagenic treatments, the ratio of purines to pyrimidines, and many others. Because of the large number of chemicals that will produce mutations and their scope of action, the opportunities for producing specific mutant alleles are great. The chemicals that will produce mutations include alkylating agents, alkaloids, peroxides, formaldehyde, nucleic acid related substances, nitrous acid, and even pure oxygen. Not one chemical group that is common to all of these chemicals confers mutagenic ability upon them. Furthermore, closely related chemicals within each group may differ widely in their ability to cause mutation. These chemicals have been tested only to a very limited extent on higher plants, and reports of combined treatments of chemical mutagens with or without ionizing radiations are scarcely to be found anywhere in the literature. Yet the literature is rich with reports of mutation studies with microorganisms. This latter literature is full of promising leads and theories that should be tested on higher plants and animals.

CHROMOSOME ENGINEERING

EARLIER in this article, the uses of mutations in breeding were listed under six headings, as proposed by MacKey. All of these headings fall into two broad groups: (a) the creation of new genes and (b) chromosome engineering. Most of the discussion thus far has concerned the creation of new genes and only incidentally the techniques of chromosome engineering—a process in which entire chromosomes or parts of chromosomes are transferred from one cell to another.

Chromosome engineering is a very fruitful technique for plant and animal improvement. Plant breeders and, to a limited extent, animal breeders use this technique daily in their breeding programs. Species hybridization and induced polyploidy are good examples of that use. With the judicious use of mutagenic agents, however, chromosome engineering can become a much more refined and subtle method of plant breeding. As examples of this, the small segments of chromosomes bearing genes for leaf rust resistance were transferred from the wild grass *Aegilops umbellulata* to cultivated wheat by Sears (*19*). Elliott (*8*) used a slightly different procedure and transferred a chromosome segment bearing genes for stem rust resistance from *Agropyron elongatum* to cultivated wheat. Chapman, *et al.*, used a combination of chromosome engineering and gene mutation to produce a new variety of crown rust-resistant oats (*5*).

Sections of chromosomes can be manipulated to a far greater extent than heretofore thought possible, even though the detailed techniques, of course, for many such possibilities are not known today. However, with the wide range of mutagens now available, such manipulation should be feasible in the near future.

Another form of chromosome engineering is that of increasing the success of interspecific hybrids. In 1952, after pollen irradiation, hybrids were reported from crosses of *Avena* species that normally were incompatible (*15*). More recently, an increased frequency of hybrids between *Lolium perenne* and *Festuca pratensis* (*16*) and hybrids between *Brassica oleracea* and *B. nigra* (*6*) were obtained with the use of ionizing radiations. This technique seems to have merit for possible use in plant breeding.

Another form of chromosome engineering is that of diploidizing polyploids. Many of the cultivated crops are polyploids with duplicate genes in the different genomes. From a theoretical point of view, if these genes were different alleles, then a permanent expression of heterosis might be expected. This hypothesis has not been proven yet, but data (*4*) are being collected to test it. If the data support the hypothesis and significant hybrid vigor can be obtained, then the diploidization of polyploids may become an outstanding technique for improving our cultivated crops. Furthermore, by the use of mutagenic agents, duplication of chromosome segments can first be produced so that diploidization of these duplicate segments can contribute to higher vigor. At the present time, this is only a hypothesis. But it may prove to have significant practical value.

Other forms of chromosome engineering can be and have been postulated. Some of these have been tested. The above examples, however, are enough to indicate the great practical value that may accrue from this field of research.

Another area of plant improvement which uses

both gene mutation and chromosome engineering is that of using mutagenic agents to improve vegetatively propagated species. It is with such crops that the greatest practical results have been made. Improved fruit and ornamental strains have been produced. Other lines carrying beneficial changes are now being tested before release.

With vegetatively propagated species, chromosome aberrations, as well as gene mutations, can be propagated because they are not eliminated through meiosis. Surprisingly, a number of the induced chromosome aberrations cause phenotypic changes that can be of economic importance. Furthermore, the arrangement of tissue in layers has allowed naturally occurring mutations to accumulate in some of the tissue layers. By the process of irradiation, these mutant layers can be "uncovered" (*18*) so that they can express themselves. This means of using ionizing radiations may prove to be profitable for fruit and ornamental plant breeders.

ANIMAL IMPROVEMENT

More research has been conducted on the use of mutagens for plant improvement than on their use for animal improvement. The research results, thus far, show that the potential for animal improvement with mutagens is not as great as that with plants. There are several obvious reasons for this. Only two will be discussed.

In the first place, plants can withstand higher doses of irradiation than animals. This is very important when high mutation frequencies are being sought because induced mutation rates are usually quite low. The average induced rate per locus under standard conditions usually ranges from 2 to 25×10^{-8}/roentgen. Since most domestic animals cannot survive doses of even a few hundred roentgens, the induced mutation rates per locus per offspring is still as low as 2 to 25×10^{-7}. This frequency is too small for practical breeding programs, especially with few offspring and long generation cycles.

The second reason for what appears to be the lack of potential from the use of ionizing radiations in animal improvement is the impracticability of manipulating and treating animals and animal sperm with the ease that plants and their parts can be treated. With such treatments, the effects of radiations on seeds can be greatly modified. Very little modification can be so attained with animals.

The use of mutagenic chemicals for producing mutations in animals, with the exception of insects, has been limited, for the most part, to rodents. Results, thus far, have been promising (*1*). It is too early, however, to speculate on the practical significance of the results. But, as more knowledge is obtained on the methods of directing mutations in plants, some of it naturally will be beneficial to the animal breeder.

This article is limited to the use of mutagenic agents in improving plants and animals by mutation breeding. However, by the use of mutagenic agents, it has been possible to eliminate certain pests of plants and animals. This is a form of plant and animal improvement which was covered thoroughly by Knipling in an earlier issue of this journal (*12*).

CONCLUSIONS

The enthusiasm expressed in this report for mutation breeding is not intended to imply that mutation breeding will replace the other more conventional forms of breeding. Even when desirable mutations are induced, in many cases they will still have to be joined with other desirable gene combinations. In addition, it is expected that for many years desirable gene mutations will continue to be associated with undesirable mutations. These desirable mutations will have to be "cleaned up" by the usual methods of crossing and selection. The hope being expressed herein is that mutation breeding will become a more valuable tool for the traditional crossing and selection procedures. It would seem that mutation breeding offers great opportunities to refine many of our plant breeding techniques.

As the centers of genetic diversity are overrun by the expanding human population and the available genetic variation is lost, plant breeders will turn to mutation breeding for the genetic variation. As plant breeding programs are increased to develop new varieties for specialized conditions—such as for mechanization of harvesting for which genetic variation has never been available—plant breeders will turn to the use of mutagenic agents for creating this needed genetic variation. Unfortunately, efficient methods for doing these things are not known at the present time. Fortunately, however, because of surpluses of agricultural products and, in many cases, a

fair amount of genetic variability, there is a "breathing spell" before the new genetic variation will be needed. This writer would like to urge that during this "breathing spell," much more research effort be applied towards developing mutation breeding techniques.

In spite of the very few improved varieties that have been released as a result of mutation breeding, Gaul (*10*) cites a large number of superior mutants that have been induced in higher plants. All of this information offers great hope for mutation breeding. Specific experiments with higher plants to test certain leads and theories from the micro-organism results will be difficult to design and expensive to conduct. Yet potentially, the practical results from such experiments are of such great magnitude that we hardly dare *not* increase our research effort in this direction.

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Salmonella in Eggs and Other Agricultural Products

George J. Banwart

ORGANISMS belonging to the genus *Salmonella* have been important in both human and animal diseases for centuries. *Salmonella typhosa,* which causes typhoid fever in man, has been extensively studied for many years. *Salmonella pullorum,* the organism causing disease in poultry, has caused much concern among poultry growers and hatcherymen.

As early as 1888, salmonellae were recognized as a food-borne infection. During World War II, with its food emergencies, the widespread prevalence of the salmonellae and the resultant illness in humans were revealed.

During the early part of this century, many reports appeared concerning the classification of the salmonellae according to their agglutination reactions. Kauffmann (*10*)[1] simplified, extended, and systemized the work of previous authors. He established the Kauffmann-White classification and made possible the uniform methods of typing now in worldwide use.

The early work of determining salmonellae in food was based on clinical tests used for *Salmonella typhosa.* Newer methods for analysis of various foods were devised during and since World War II, so that the sources of salmonellae are more readily identified today than ever before.

[1] Italic numbers in parentheses refer to "Literature Cited" p. 14.

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DISTRIBUTION IN NATURE

EDWARDS *et al.* (*5*) summarized their salmonellae studies up to 1948. They reported on 12,331 cultures isolated from 47 animal species which included fowls, reptiles, lower animals, man, and from water, sewage, eggs, egg powder and various other food products. The 12,331 cultures comprised 111 different serotypes of salmonellae.

One conclusion from their data is that domestic fowl is an important reservoir of salmonellae, since over 50 percent of the cultures were isolated from fowl. The large proportion of isolates from poultry reflects the good response of diagnostic services, pathology laboratories, and pullorum testing stations in forwarding cultures for typing or in submitting results of typing poultry isolates. On the other hand, in most suspected cases of salmonellosis in large animals (hogs or cattle) a clinical diagnosis of the disease and successful treatment were usually considered sufficient, and cultures from these animals were not taken or were not made available to typing centers. Of the 6,263 cultures from domestic fowl, 4,007 were isolated from turkeys despite the fact that the population of turkeys is considerably smaller than is that of chickens and other domestic fowl. Two other important reservoirs of salmonellae are man and swine.

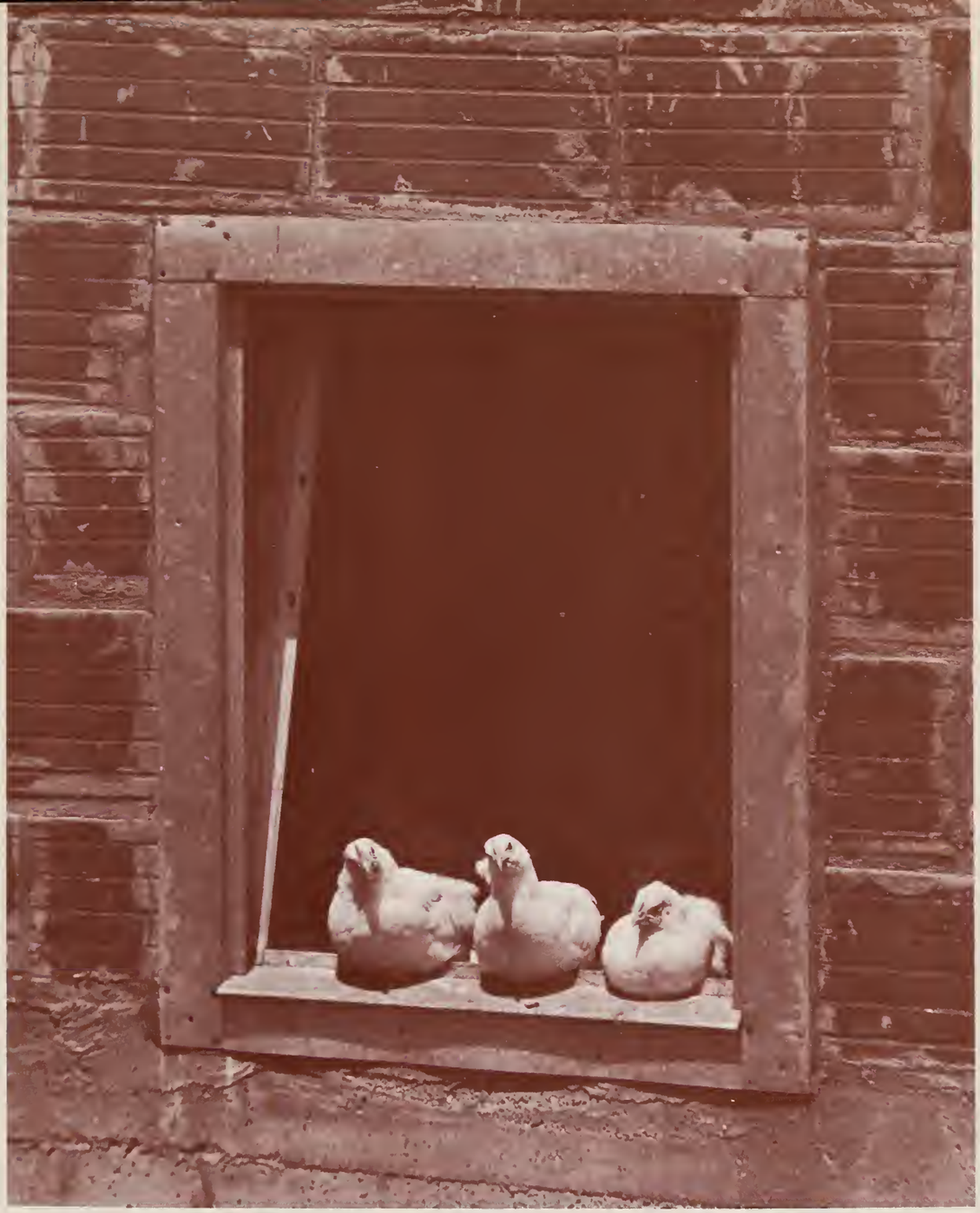
Swine as a reservoir becomes important when one considers the number of swine as compared to the number of poultry. In the data of Edwards *et al.* (*5*) swine accounted for 2,060 cultures as compared to 6,263 cultures from poultry, or approximately one-third as many cultures. The swine population on farms is approximately 60 million; that of poultry is about 875 million. Since, as mentioned previously, cultures from poultry were typed more frequently than those of swine, the swine becomes more prominent as a source of salmonellae, and the percent of swine from which salmonellae are isolated is much higher than that of poultry. Reports in 1963 from the *Salmonella* surveillance unit of the Public Health Service showed that for nonhuman sources of salmonellae, poultry accounted for approximately 47 percent, swine 22 percent, and cattle 12 percent of the number of isolates. The public health aspects of swine and cattle salmonellosis have not been emphasized as much as that of poultry or poultry products. The reason for this is that poultry products are involved with human salmonellosis more than other products. The U.S. Public Health Service (*15*) reported the investigations of 32 salmonellosis outbreaks between March, 1962 and April, 1963. Of these, 22 had a definitely established source of contamination and 14 (64 percent) of these were due to poultry.

ENTRY INTO FOODS

ONE of the many problems is to determine the main sources of these pathogens in the food products for humans. Many suggestions have been made. The data of Hinshaw and McNeil (*7, 8*) and Edwards *et al.* (*5*) showed that rodents, reptiles and wild birds harbored salmonellae organisms. It was thus postulated that these wild animals could transmit the organisms to the domestic animals, especially fowl, by contaminating feed or water. However, the idea that feed might be contaminated by salmonellae has led to another field of investigation.

Walker *et al.* (*16, 17*) reported the analyses of 5,419 samples of feed ingredients and finished feeds for salmonellae. Of 175 samples of meat products used in feeds, 29 were positive for salmonellae (16.6 percent). Other feed ingredients showed that 14.4 percent of samples of marine products and 5.1 percent of vegetable products contained salmonellae. Products made from bone, such as bone meal, were very often contaminated; 76.7 percent of these samples were positive for salmonellae. In a review paper, Morehouse and Wedman (*14*) showed that of some 4,480 samples of feed ingredients, 643 or over 14 percent contained salmonellae. With data such as these showing the incidence of feed contamination, perhaps wild animals are not an important source of salmonellae. Do rodents contaminate the feed with salmonellae or does the feed contaminate the rodents?

Is the contamination of animal feed by *Salmonella* important? The Public Health Service apparently believes that feed contamination is of primary concern. The investigation of the interstate epidemic of hospital-associated *Salmonella derby* infection in 1963 centered on food products. Although other foods were found to contain *Salmonella,* the possibility of eggs as a source of infection was investigated. Of 235 dozen eggs, 5,400 samples of chicken droppings and 58 samples of feed, positive isolations were obtained from four samples of



cracked eggs and one sample of feed. Several workers have been studying the carryover of salmonellae from infected feed into the eggs laid by the hen. All recent studies have shown that no salmonellae have been found in eggs laid by hens eating feed that contains these organisms or even by inoculation of the birds with live organisms. Many reports in the literature have shown that *Salmonella pullorum* reactor hens do lay eggs infected with this organism; the percentage of infected eggs varies from 0 to 33.7 percent. Gibbons and Moore fed *S. pullorum* and *S. bareilly* to hens. They isolated *S. bareilly* from the shells of 3 of 37 eggs and *S. pullorum* from the egg contents. Thus, it is apparent that *S. pullorum* might be deposited inside the egg by the hen; it is questionable if other salmonellae contaminate the interior of the egg in this manner. In the hospital outbreak of salmonellosis, only cracked eggs were cited as being the cause.

Since eggs are cracked by many different means, should all cracked eggs be condemned? Should only eggs that are cracked in the nest be condemned and those cracked at the processing plant be acceptable for use? Actually, what is a cracked egg? Are only obviously cracked eggs considered in this category or should cracked eggs include checks that can only be seen by use of a candling light? Where do the cracked eggs become contaminated? If

the egg is cracked in the nest and the hen is a shedder of salmonellae, contamination might occur. Used egg cases have been found to contain salmonellae. If the shell is cracked, it is conceivable that the organism from casing material could invade the egg. Perhaps the largest reservoir of salmonellae is the human population. During handling of cracked eggs at the processing plant, they might be contamined from human sources. Thus the source of salmonellae in eggs can be quite varied.

The role of shell eggs as a cause of salmonellosis in humans has become more prominent in recent years, although previously the *Salmonella* problem was focused on egg products. The sources of these pathogens in egg products are many. Any source of contamination of shell eggs can also serve as a contamination source for liquid or frozen egg products. Besides these sources, the human reservoir can more readily contaminate the edible portion of liquid eggs than shell eggs. Only a few shell eggs infected with salmonellae can contaminate an entire vat of liquid eggs. With the use of tanks holding 30,000 or more pounds of liquid, contamination of a large amount of liquid or dried eggs is quite possible. When the eggs are drawn-off for freezing or drying, the contaminants can be well distributed throughout several containers of product.

PREVIOUS RESEARCH

MUCH work has been done by various research groups to eliminate salmonellae from egg products. Pasteurization of liquid egg was found to be beneficial in destroying salmonellae. Winter and Stewart (*18*) reported that 140° F. for 2.6 minutes killed most *Salmonella* species in liquid whole egg. Goresline *et al.* (*6*) observed that heating to 140° F. and holding for 3 minutes would kill any salmonellae present in the liquid egg.

Although pasteurization can be used to advantage, there have been problems associated with using this procedure. Many small egg products companies cannot afford the expense of pasteurizing equipment, since their volume does not warrant the cost. Other companies have not been technically staffed to understand and utilize the pasteurizer as it should be used. Some processors have argued that heat treating the egg reduces the baking performance of the product in cakes. Research reports do not indicate a loss of performance, but often the results in a laboratory cannot be reproduced under plant conditions. Even if a product is pasteurized and salmonellae destroyed, there is always the possibility of recontamination of the product between pasteurization and packaging. Another problem with pasteurization is that because of the low temperature of coagulation, the process is not effective for destroying salmonellae in egg white.

Since egg white has been found to contain salmonellae, and since this product is used in foods such as meringues and icings which are not heated enough to destroy these organisms, a method to eliminate these pathogens was needed. Banwart and Ayres (*2*) reported that by holding dried albumen at elevated temperatures (120°–158° F.), salmonellae in the product could be destroyed. Banwart and Ayres (*3*) further observed that raising the pH of the liquid albumen to 8.5 or higher destroyed salmonellae. Bergquist (*4*) received a patent to destroy salmonellae in egg white. His method consisted of adjusting the pH to 7.5 or 9.0, heating the liquid to 130° C. for 1 minute, and, after drying, storing the product at 120° F. for 7 days. This procedure is similar to previously reported information except that the liquid is heated to 130° F. for 1 minute.

A temperature of 130° F. has not been effective in destroying salmonellae in other food products. The thermal death time curves for *S. senftenberg* in chicken a-la-king or in custard showed that approximately 2½ hours at 130° F. was necessary for destruction (Angelotti *et al., 1*). Other data by these authors showed that an inoculation of 10 million cells of *S. senftenberg* in custard required between 500 and 600 minutes exposure to 130° F. to cause complete destruction of the organisms. Thus, most of the destruction of salmonellae in albumen is most likely due to the pH effects and storage of the dried product at 120° F.

The processes now in use by industry to eliminate salmonellae from foods, particularly eggs, are not entirely effective. Dried whole egg produced from pasteurized liquid has been involved in outbreaks of salmonellosis. Specially produced dried egg albumen has been found to contain salmonellae. The present processes need modifications, or new methods need to be explored to eliminate salmonellae from the liquid or dried product. Some possibili-

ties might be ultraviolet light, addition of hydrogen peroxide or other chemicals, or radiation treatment. The use of hydrogen peroxide is being used commercially in a round about way. If, during removal of glucose to stabilize the eggs, glucose oxidase is used, hydrogen peroxide is allowed to be added as the oxygen source for the reaction changing glucose to gluconic acid.

Food may not contain salmonellae until the consumer contaminates the product during preparation prior to eating. It is unfortunate for the egg processor that his product allows the prolific growth of these pathogenic organisms. Not only that, but egg products are often eaten with little or no cooking. Even though pork has been found to contain salmonellae, this meat seldom causes outbreaks of salmonellosis. The prevalence of trichinosis in pork products has made the consumer aware that thorough cooking is necessary before eating. Consumer practices in handling food products are the major cause of salmonellosis. McCullough and Eisele (*11, 12, 13*) found that several thousands of salmonellae were needed to cause symptoms of food poisoning in human volunteers. Food products are not ordinarily contaminated to the degree that would cause illness. But allowing food to remain at temperatures that permit growth of pathogenic organisms that might be present can increase the population to the point where food poisoning can result. Consumers should be educated in the proper handling of foods, especially those that are more likely to contain salmonellae.

NEEDED RESEARCH

THE problem of *Salmonella* in agricultural products is centered on poultry and poultry products. There are several avenues of research that have been studied and need further study. The Public Health Service through their *Salmonella* surveillance is doing a marvelous job of reporting sources of infection. Since these pathogenic organisms are so widespread, it appears impossible to control all the possible sources of contamination. However, each potential infectant should be studied and information acquired to control the dissemination of the salmonellae.

Perhaps better disinfecting agents are needed on the farms, and their use supervised so that they are effective. Pelleting of feed has been found to reduce or eliminate salmonellae from this product. The heat treatment during pelleting is responsible for destroying the organisms. Perhaps all feeds could be treated, if a good, economical system were developed.

In processing plants, sanitation alone does not insure that salmonellae will not infect the product. For shell egg and egg product plants, perhaps an acceptable washing procedure is needed and each egg washed and sanitized before being processed. Egg breaking machines are available that will wash and chlorine spray every egg prior to breaking. There is no direct contact between the food product and man. There is no information regarding the effectiveness of this machine to produce a product devoid of salmonellae.

Considering all the potential sources of salmonellae contamination, the most logical place to attempt to control salmonellae in foods is after the food is packaged, assuming, of course, that the processor follows good sanitation and refrigeration procedures. Fumigation has been used to sterilize spices. This might also be effective with other products, such as eggs. Further work needs to be done regarding this type of disinfecting agent. Another possibility is storage of the product at elevated temperatures such as is done with dried egg white. There is no doubt that processes to destroy salmonellae can be developed. However, these procedures for destruction of bacteria must not damage the food product. For example, eggs are used in other foods such as cakes primarily for their functional properties. It is therefore necessary that the process used to destroy salmonellae does not impair the functional properties of eggs. This aspect makes the development of salmonellae control much more challenging. There must be teamwork between the bacteriologist and food technologist to develop acceptable processes.

Before effective control measures can be successful, a method or methods to enumerate the salmonellae are needed. There is much information in the literature regarding media and methods for detecting and isolating those organisms. Since many laboratories are concerned with salmonellae, several methods for detection have been devised. No doubt they all are satisfactory to some degree, but none of them are completely without error.

The Institute of American Poultry Industries (*9*) listed 12 methods used to detect salmonellae in egg

products. There are probably numerous others used by various laboratories. In the list were three methods used by each of three government agencies. There has been an increasing desire to set up an international method for *Salmonella* isolation. We can't even agree in this country on a method acceptable to everyone. What is probably needed is a *Salmonella* Convention where all the methods and media can be discussed and some conclusions made. Then, after a year's trial in their own laboratories, scientists could meet again and attempt to develop methods of analysis acceptable to everyone. This type of meeting would be needed each year or two to revise the method with any new developments that might be made. Quite possibly, a method will need to be devised for each type of food product. Reports in the literature have indicated that a method developed to more readily isolate salmonellae from egg white did not increase the isolations from whole egg or egg yolk. Each type of food product has characteristics that might require a revision in a particular method.

Further research is needed to help solve the problems associated with salmonellae in agricultural products. Answers are needed so that there is control of salmonellae in feeds, growing animals, in the processing plant, and up to the time the food is eaten by the consumer. More efforts should be made to educate the consumer in the proper handling of foods. Completely satisfactory methods to determine salmonellae in food products are needed—primarily methods acceptable to all scientific personnel involved in salmonellae analysis.

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Rural Education in Transition

RESULTS of a Louisiana study of trends and patterns in rural education clearly have applications in other States and areas where similar conditions prevail, particularly in terms of public school planning for the future. Findings of the study have been documented and published by Marion B. Smith and Alvin L. Bertrand, Professor Emeritus and Professor, respectively, Departments of Sociology and Rural Sociology, Louisiana State University.

The authors point out that the rural segment of the population is not sharing in the overall population growth of the State. This phenomenon—not unique to Louisiana—is accounted for by the technological innovations that have reduced labor needs in agriculture and related rural occupations. The implications for rural education of a static population are not too clear, state the authors, but certain observations can be made.

"In the first place, additional teachers and additional classrooms will not be serious problems in the foreseeable future, as will be the case in urban areas. Nor will there be an alarming deficit in the quality of instruction if one is to judge according to the trends which indicate that the training and experience of rural teachers have improved through the years and is now comparable to that of urban teachers.

"Secondly, trends in enrollment indicate that school attendance is no longer the problem (legal or otherwise) in rural Louisiana that it was a few years ago. Apparently, efforts to get children in school have been successful in even the most isolated places.

"In the third place, it is apparent that rural schools serve a great majority of students who will not find their life's work in agriculture, and who will not live out their lives in rural areas. The significance of this fact for high school curriculum and for the general philosophy of education in rural areas is self-evident. It represents an important and immediate challenge to curriculum planners.

"Finally, there is an overall implication that stems from the fact that both the population and the sociocultural patterns are changing rapidly in the State. As levels of living, educational levels, communication, and transportation improve, it is inevitable that attitudes and values toward education will change. Persons who share a concern over rural education must familiarize themselves with these trends if they are to plan wisely for the future."

From: Louisiana Agricultural Experiment Station Bulletin No. 576, "Rural Education in Transition—A Study of Trends and Patterns in Louisiana."

FERTILIZER NITROGEN

Franklin E. Allison

How Its Efficiency is Affected by Soil Microorganisms

THE efficiency with which nitrogen fertilizers are likely to be utilized by crops grown under various conditions can now be predicted with considerable accuracy if the conditions are well defined. This development is largely the result of intensive research in recent years on the activities of the soil microorganisms that are responsible, directly or indirectly, for most of the nitrogen transformations occurring in soils.

Types of Organisms in Nitrogen Transformations

SOIL microorganisms responsible for nitrogen changes include bacteria, actinomyces, and fungi. Like all living organisms, they must have a source of energy to live and multiply, and the abundance of this energy supply is usually the main factor that determines growth. On the basis of their energy source, they are classified as either heterotrophic or autotrophic.

Heterotrophs

THESE microorganisms, which comprise the bulk of the soil population, both in number of species and amount of growth, live on organic compounds synthesized by plants and animals. Since animals obtain their food by consuming plants, it is obvious that the ultimate source of energy in both instances is sunlight. The soil microorganisms oxidize the plant and animal tissues to carbon dioxide, water and various byproducts. In the decomposition of plant materials there is a nearly complete utilization of the readily oxidizable organic constituents. Much of the liberated energy is utilized inefficiently. The nitrogen originally present in the decomposing plant materials that is not needed by the microorganisms for synthesis of their own cells is released largely as ammonia.

Autotrophs

AUTOTROPHIC microorganisms do not obtain their energy from organic compounds but by oxidizing certain elements or simple inorganic substances. These include chiefly ammonia, nitrite, hydrogen, and oxidizable compounds of sulfur, carbon, iron and manganese. They obtain their carbon from carbon dioxide. The autotrophs do not grow rapidly and form large masses of growth as do many heterotrophs. So far as nitrogen is concerned, the two most important and very beneficial genera are *Nitrosomonas,* which oxidizes ammonia to nitrite, and *Nitrobacter,* which oxidizes the nitrite further to nitrate. Since these organisms do not depend on organic matter for energy, they may grow well on very poor soils, although they usually function better on high-humus soils because such soils have a higher buffering capacity and contain more carbon dioxide and inorganic nutrients.

SOURCES OF NITROGEN FOR PLANT GROWTH AND RECOVERIES

AMMONIA and nitrates are the chief forms of nitrogen assimilated by crops. Nitrite is also utilized but it is toxic at comparatively low concentrations. It is usually present in soils only in traces, although heavy applications of ammonia fertilizers may retard nitrite oxidation by *Nitrobacter* and allow it to accumulate. Some simple forms of organic nitrogen, such as amino acids and urea, may also be utilized directly. Legumes nodulated with effective bacteria can, of course, grow normally on atmospheric nitrogen.

The merits of ammonia as a direct source of nitrogen for plants have been discussed frequently. It has been shown that many microorganisms and higher plants will, under ideal conditions, absorb ammonia preferentially over nitrate in experiments lasting a few minutes or hours. In longer experiments, however, where growth metabolism is involved, this difference seldom occurs. If the supply of basic elements is so low that considerable acidity develops in the culture medium, nitrate is likely to be the preferred source of nitrogen. In other words, nitrate is a more "fool-proof" nitrogen source for most plants than is ammonia, although both sources are nearly equally good when used under proper conditions.

Nitrogen recovery in crops, including roots, seldom exceeds about 75 percent under the most ideal conditions, as in greenhouse pots where leaching is prevented. Nitrogen tracer experiments have shown that usually half or more of the unrecovered nitrogen remains in the soil, whereas the remainder escapes as gases. Under field conditions, where leaching may occur, a recovery of 50 to 65 percent of the nitrogen in the crop, including roots, is considered very good. If only the harvested portion of the crop is considered, the recovery may be

much less than 50 percent. In longtime field experiments, recoveries in harvested crops of 30 to 50 percent of the added fertilizer nitrogen have been common.

CONDITIONS THAT DELAY NITROGEN RECOVERY

ADDITIONS of nitrogen often fail to give the expected rapid increases in plant growth and crop yields even though losses as gases, or in the drainage waters, are kept at a minimum. This disappointing response to nitrogen may be due to a variety of causes but the main reasons are likely to be either: (a) low availability of the added nitrogen source, (b) nitrogen immobilization by microorganisms that live on crop residues or in the rhizosphere, or (c) unfavorable conditions for plant growth, including deficiencies of other essential nutrients.

Additions of slowly available forms of nitrogen

MOST of the nitrogen now added in fertilizers is in readily available forms. In the past it was common to add considerable byproduct organic nitrogen, such as slaughterhouse wastes, seed meals and sewage wastes. But in recent years most of this type of organic nitrogen has been diverted to animal feeds, except that type unsuited to feed use. More recently, other forms of nitrogen, especially urea-forms, have been used in special fertilizers. These specially-prepared compounds release their nitrogen gradually during the growing season. It is also possible that certain inorganic forms of nitrogen, such as magnesium ammonium phosphate, may be used in the future. Nitrogen in this compound is utilized more slowly than is nitrate, or the more commonly used forms of ammonia, because of its low solubility. Any appreciable delay in uptake of fertilizer nitrogen by plants introduces chances for losses to the air or drainage waters.

Addition of nitrogen-deficient crop residues

THE addition of carbonaceous crop residues, such as cornstalks and straw, results in the tieup of considerable nitrogen. Such materials, which have nitrogen contents of about 0.3 to 1.0 percent (carbon-nitrogen ratios of 130 to 40), are decomposed by microorganisms that contain 2 to 12 percent nitrogen (carbon-nitrogen ratios of 20 to 4). Although 50 to 90 percent of the carbon of the plant materials is released as carbon dioxide during decomposition, there is still a large nitrogen deficit. In other words the nitrogen in the crop residues is inadequate to build the microbial tissues; hence the microorganisms make up the deficit by using as much as needed of the available soil or fertilizer nitrogen.

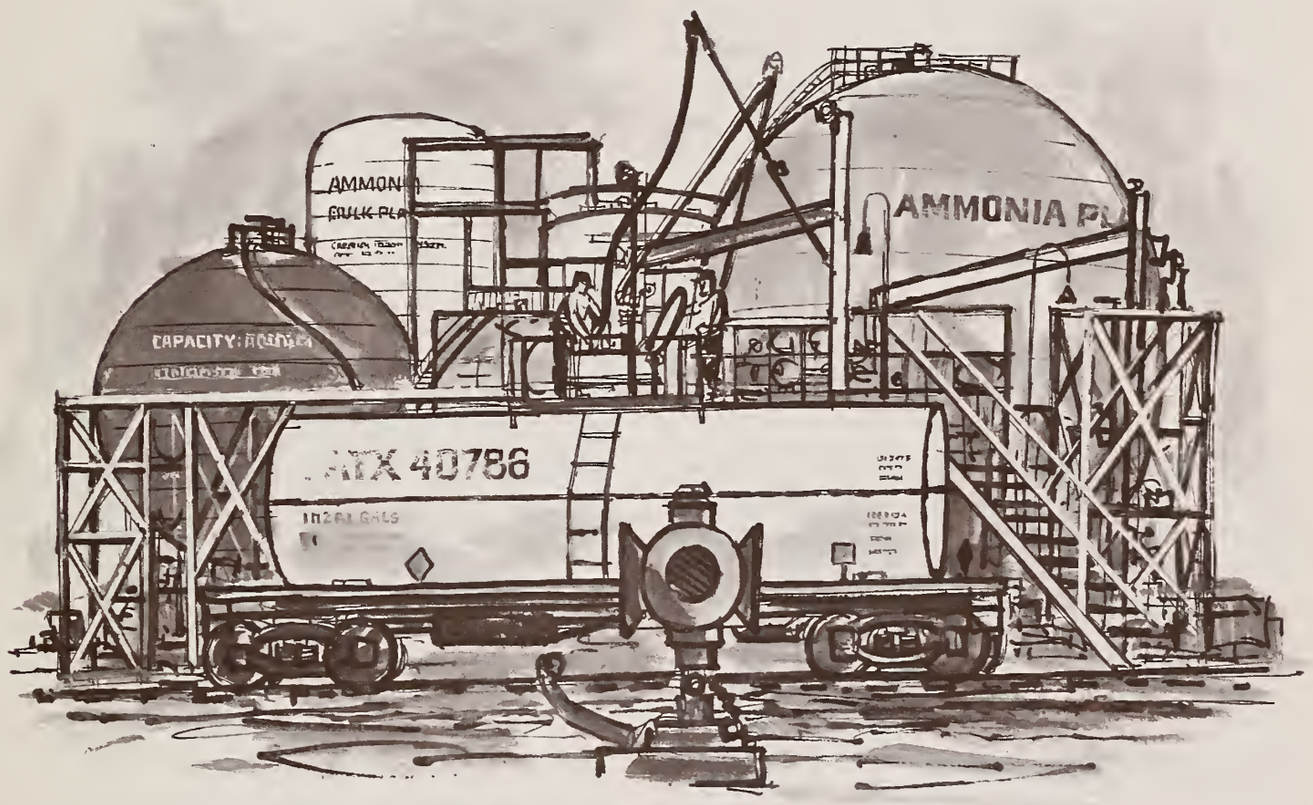
Nitrogen immobilization starts immediately after straw or other materials are added to moist soil, and under favorable conditions is likely to reach a maximum after about three weeks. If highly available energy sources, such as sugar, are added, maximum immobilization is realized within two or three days; for materials less readily available than straw the period may be two months or longer. As the ease of decomposition of the highly carbonaceous plant substance increases, the quantity of nitrogen immobilized likewise increases.

The immobilized nitrogen is gradually and slowly released during the period following maximum immobilization. It is not all released, however, during the growing season. The resistant residue becomes a part of soil humus, some of which remains for many, many years.

The immobilization of available nitrogen in soils also proceeds very slowly and continually in the absence of crop residues. Simultaneously, there is a reverse movement of similar or slightly greater magnitude in the conversion of soil nitrogen into ammonia and nitrates. These normal movements of nitrogen are sometimes designated as biological interchange. Recent greenhouse experiments with tagged fertilizer nitrogen emphasized these changes. As the amount of fertilizer nitrogen was increased, the amount of soil nitrogen assimilated by the crop increased. Soil analyses showed that comparable amounts of tagged nitrogen were left in the soil after harvesting the crop. Such findings could have been due only to the mineralization-immobilization activities of the soil microorganisms. These findings, still under active study, are of much scientific interest, but may be of minor importance so far as efficiency of use of nitrogen in crop production is concerned, since tagged and untagged nitrogen merely exchanged places.

Since soil humus is universally recognized as a very valuable material, attempts have been made to hold more of the added plant carbon in the soil by adding excess fertilizer nitrogen. Such attempts

have been unsuccessful because the retention of plant carbon in the soil during the initial stages of decay primarily depends upon the properties of the plant substance—that is, on its resistance to decay and not upon the level of nitrogen present. Under cropping conditions it is true that the addition of abundant nitrogen to a soil may tend to keep the soil humus content at a higher level than where nitrogen is deficient, but only because the nitrogen increases crop growth, and larger crops mean larger additions of crop residue carbon to the soil.



Immobilization of nitrogen by rhizosphere microorganisms

EACH plant root or root hair is surrounded by thousands or millions of microorganisms that feed upon the organic compounds secreted or excreted by the plant roots and on sloughed-off root cells. The total amount of nitrogen immobilized by these microorganisms is determined by the amount of readily oxidizable carbon released by the plant roots. If the carbon-nitrogen ratio of the released substances is narrow, the nitrogen immobilized is chiefly nitrogen that was previously taken up by the plant. If the ratio is very wide, the organisms will obtain a portion of their supply directly from any available soil or fertilizer nitrogen remaining in the soil.

Data are not available to show the extent of nitrogen immobilization by the rhizosphere organisms of different crops but undoubtedly the tieup of nitrogen by these organisms is greatest for the plants that are normally rather low in nitrogen and have masses of fine roots, especially the grasses. The immobilized nitrogen in the cells of the rhizosphere microorganisms is slowly released when they die and decompose.

Unfavorable conditions for plant growth

DELAYED utilization of fertilizer nitrogen by a crop may also be expected if the crop is grown under unfavorable conditions. This fact, although quite obvious, is often not fully appreciated. The unfavorable growth conditions may be the result of a long list of factors typical of which are: deficiency of other essential elements, unfavorable soil reaction, excess or deficient moisture, poor aeration, poor physical condition of the soil, hardpan, unfavorable temperature, or poor crop variety. For most crops it is especially important that growth conditions be as ideal as possible during early growth. A stunted seedling seldom fully recovers later. It is also important that, so far as possible, the plant be supplied with adequate, but not excessive, amounts of nitrogen as needed. If the nitrogen is not utilized by the crop to which it is applied it is unlikely to be utilized efficiently by the crops that follow.

CONDITIONS LEADING TO LOSS OF NITROGEN

Since delayed assimilation of nitrogen commonly results in lower and lower efficiencies of nitrogen utilization, largely because of loss from the soil, it is well to consider in some detail the mechanisms and magnitude of these losses. They can be avoided, at least in part, only if the channels of loss are fully understood. The main factors involved in leaching losses are now well understood. But losses in gaseous forms may occur in a variety of ways; these are still not fully understood and usually cannot be evaluated quantitatively.

Loss of nitrogen through leaching

THE only form of nitrogen ordinarily found in quantity in soil drainage waters is nitrate. This form not only is water soluble but also is not adsorbed and retained in soils as are many other nutrients. This contrasts with water-soluble ammonia which is held very tenaciously as the ammonium ion by clays. A soil must be extremely sandy for more than traces of this form of nitrogen to be removed by leaching, assuming, of course, that an excessive amount was not added. Urea, a water-soluble compound now commonly supplied in fertilizers, decomposes so readily into ammonia in soils that little of this nitrogen escapes as urea. Both urea and ammonia sources are rapidly oxidized biologically to nitrite and nitrate, and these compounds are subject to leaching.

Accurate data showing the magnitude of nitrogen loss by leaching under various soil conditions are either scarce or unavailable. This is due chiefly to the difficulty of measuring such losses. About the only way to make such measurements is with lysimeters where the soil is maintained in columns and the leachate is collected. Obviously, such experiments do not accurately duplicate field conditions and they have been subject to considerable criticism over the years. The determination of leaching losses is also confounded by the simultaneous loss of unknown amounts of nitrogen as gases, and by immobilization of added nitrogen by the soil microflora. A study of all of the facts shows that leaching losses vary widely and are often quite large. In dryland regions such losses are near zero, whereas in sandy soils of the humid regions they may amount to half or more of the added fertilizer nitrogen.

The importance of considering rainfall-evapotranspiration values for each location has been emphasized recently. If the water lost by evapotranspiration equals or exceeds the amount of rainfall that enters the soil during any period of a few days, it is obvious that leaching cannot occur unless the soil water was much above field capacity initially. Graphs showing rainfall-evapotranspiration values, usually plotted on an average monthly basis for 20 years or more, have been published for several regions in the eastern states. In nearly all cases these graphs show that for soils of medium texture there is little or no leachate during the period of approximately May to October. This would not be true during certain periods of abnormally high rainfall or for very sandy soils. It does hold, however, for most soils for about 9 out of 10 years. In order to minimize leaching of nitrates, then, it is necessary to keep the nitrogen at a low level during the fall and spring months, and also during the winter if the soil is not continually frozen. Fortunately, nitrogen can be added during the main crop growing period without much chance of loss in the drainage waters.

In irrigated regions leaching losses can be kept to a minimum by manipulating the times of fertilizer application and water additions.

Loss of ammonia-nitrogen by volatilization

AMMONIA losses from soils by volatilization are small under most conditions but may, in a few instances, be as high as 20 percent of that added. The losses are likely to be negligible from soils having pH values of less than 7 except possibly where the exchange capacity is extremely low, or where the ammonia addition is high enough to raise the pH locally in macropores. As the pH is raised, or the soil undergoes drying, the chances for loss of ammonia increase, especially if it is present near the soil surface. If animal manures, or readily decomposable plant materials that have nitrogen contents above 1.5 to 2.0 percent, are allowed to decompose on the soil surface some ammonia may escape. The amount so lost increases markedly as the nitrogen content of the decomposing substances increases. The loss by volatilization may be entirely prevented if the materials are incorporated into the soil before biological action starts. This holds true even for alkaline soils.

Losses of ammonia from urea applied to grasses or to soil surfaces have probably been overemphasized in recent years. Such losses certainly can occur if the enzyme urease, produced by microorganisms and higher plants, comes in contact with urea and splits off ammonia. Such losses can be prevented by mixing the urea with the soil.

Loss of nitrate- and nitrite-nitrogen through denitrification

BACTERIAL denitrification, involving the release of nitrous oxide or free nitrogen gas from nitrates or nitrites, has long been recognized as one of the chief channels of loss of nitrogen in gaseous forms from soils. It was formerly thought that such losses were limited largely to manure piles or waterlogged soils where there is an almost complete absence of oxygen. More recent work has shown that losses can occur, at least to some extent, where some oxygen is present in the soil atmosphere. Any tendency for the formation of anaerobic pockets in the finer-textured soils also increases the chance for losses. The bacteria that release nitrous oxide and elemental nitrogen are heterotrophs that require oxygen for their growth. They use the oxygen of the air preferentially but if this is absent or inadequate, they can obtain the element from the oxidized forms of nitrogen. Expressed in biochemical language, the oxygen serves as a hydrogen acceptor. Careful farm management can usually keep denitrification losses at a low level except during periods of abnormally high rainfall and in poorly drained soils.

Loss of gaseous nitrogen through chemical reactions involving nitrous acid or nitrite

THE most recent research has shown that nitrous acid or nitrites are so unstable and reactive that large losses of gaseous nitrogen from soils may be expected under many conditions where these compounds are formed in quantity, and not rapidly oxidized to nitrate. If certain organic nitrogen compounds or ammonia are also present the nitrogen losses are likely to be greater than in their absence, since reactions can occur between the two types of compounds. Some of the possibilities will be considered.

Nitrous acid, itself, is reasonably stable in neutral or alkaline soils where it exists as salts. In acid soils, however, it is not stable and nitric oxide may be released. This oxide may be volatilized in part, but most of it is likely to be adsorbed by the soil and then undergo oxidation by soil air to form nitrate. Exact data on the extent of volatilization of the oxide are not available but there is no reason to expect that a large percentage of the nitric oxide escapes as gas except perhaps where there is a decided delay in nitrification.

The possibility of nitrous acid reacting with alpha amino acids (so-called Van Slyke reaction) to form free nitrogen, has been much discussed. Soil biochemists now generally agree that this reaction is of very minor importance in soils, contrary to some earlier reports. Not only are free amino acids nearly absent from soils, but any nitrous acid present is more likely to be decomposed to nitric oxide, or to be nitrified than it is to react with amino acids.

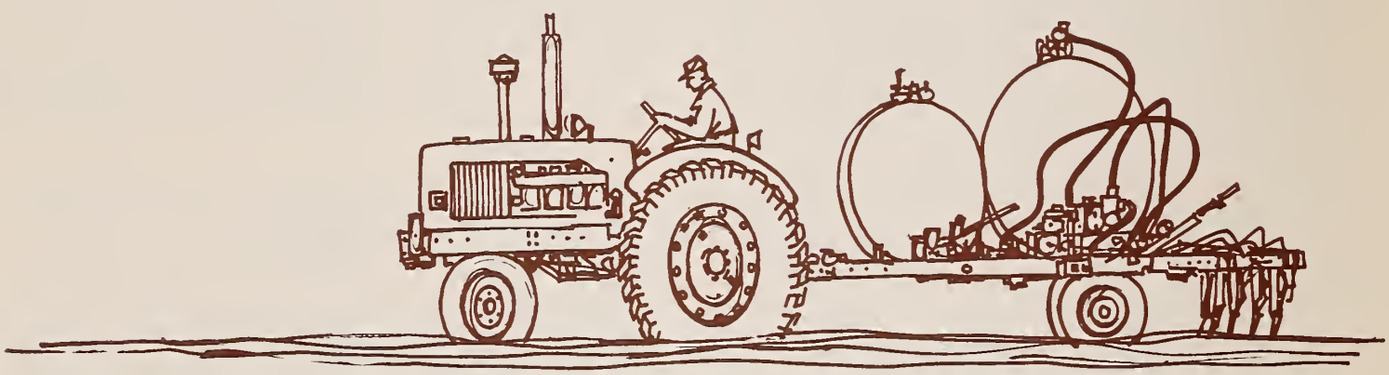
A more important channel of loss of nitrogen gas than via the Van Slyke reaction may be via ammonium nitrite. This compound can form at any soil pH where either nitrous acid or nitrite ions are present in appreciable concentrations together with ammonia. Ammonium nitrite is somewhat unstable, and on decomposition releases gaseous nitrogen. The reaction is catalyzed by hydrogen ions but can proceed slowly in an alkaline medium.

CONDITIONS THAT FAVOR HIGH RECOVERY OF NITROGEN IN HARVESTED CROPS

THE above discussion has emphasized that nitrogen is an element that tends not to stay long in an unchanged form in soils unless it becomes tied up in the more resistant parts of soil organic matter or humus. This is due chiefly to the activities of a wide variety of microorganisms that are constantly decomposing nitrogen-containing materials, oxidizing some of the released nitrogen, and immobilizing other portions. Strictly chemical changes apart from microbial activities are in the minority. If the nitrogen is added in nitrogen-rich plant materials, for example, these plant tissues are quickly decomposed and much of the nitrogen is liberated as ammonia, or sometimes as elemental nitrogen. This ammonia, or added fertilizer ammonia, may then be either oxidized or assimilated by microorganisms or higher plants. Nitrogen supplied as nitrate may be either assimilated, leached out, or reduced to gases. Whenever any of these microbial transformations occurs there is always a chance for losses.

It is evident from these facts that the best way to insure a high recovery of fertilizer nitrogen in the crop is to apply it in readily available forms at such times and amounts as the crop is able to assimilate it quickly. This minimizes side reactions and possibilities of loss such as would be involved if, for example, the nitrogen were applied to a green manure crop and a second crop planted after turning this under. Even more inefficient would be the addition of water-soluble nitrogen in large amounts to freshly-seeded land during very rainy periods. Likewise, fall applications of nitrogen in a warm climate would not favor high recoveries of this nitrogen in a crop grown the following season.

Practical considerations cannot, of course, be overlooked in nitrogen fertilization practices. There are times and places where the agronomist is not justified in striving for the very highest possible efficiencies in nitrogen use. Fertilizer nitrogen is now in ample supply at reasonable costs, whereas labor may be scarce and comparatively high priced. Under such conditions the planting of a winter cover crop, for example, just to save a few pounds of nitrogen would obviously not be justified unless other factors, such as erosion control, were also important. In most cases, however, the fertilization and cropping programs can be correlated satisfactorily to yield a high level of efficiency in nitrogen use.



RESEARCH NEEDS

ALTHOUGH much has been learned in recent years about nitrogen transformations in soils and how to control them so as to increase nitrogen-use efficiency, it is also true that new problems have arisen, partly as a result of the introduction of new

fertilizer materials and methods of use. In addition, many of the old problems, especially those involving nitrogen losses from soils, have been solved only partially. Much more basic data are needed on what happens to nitrogen in soils. Some examples are as follows:

1. Anhydrous ammonia is now a major source of nitrogen for crop production. When this is injected into soil it partially sterilizes the soil at the point of injection. Nitrification may be delayed, the pH is raised, and some ammonia may be lost as gas from the coarser textured soils under some conditions of use. The needs for further bacterial, chemical, and plant research are obvious.

2. Urea, another important source of nitrogen, is readily decomposed to ammonia when it comes in contact with the enzyme urease. This enzyme is produced and released by many higher plants and microorganisms. How best to minimize the losses of the ammonia under practical conditions of use needs further study.

3. The use in recent years of slowly available forms of nitrogen, especially urea-formaldehyde products, has also introduced problems involving bacterial transformations. Along with the reduced rate of release of the nitrogen in available forms is also the matter of efficiency of use. What percentage of the added urea-formaldehyde is eventually utilized by the crop under a variety of soil and plant conditions?

4. Considerable emphasis is now being given by fertilizer manufacturers to the possibility of coating fertilizer materials to prevent quick release of nitrogen. Neglecting initial costs, are such materials likely to be generally superior to uncoated materials? Present information is inadequate to show if such a method of feeding crops is a sound and desirable practice.

5. Soils that contain an abundance of illite, vermiculite, and montmorillonite have been shown to fix considerable ammonia in the crystal lattices of these minerals. Some of this ammonia is held so tightly that neither bacteria nor higher plants can obtain it readily, if at all. In spite of much recent work on this subject we still are unable to state with certainty how much of this fixed ammonia is released from various soils and at what rate. Since fixed ammonia is not lost by leaching or as gas, the fixation process may be desirable under some conditions. More basic and field data are needed.

6. Further bacterial denitrification studies are needed that will show if this mechanism of loss is important in soils that are commonly considered well aerated. Do such soils contain anaerobic pockets, or micropores, where oxygen diffusion inward from the air is slower than its rate of consumption inside? Do bacteria release appreciable amounts of denitrifying enzymes that are active in spaces that are too small for bacteria to enter?

7. A thorough study of the reactions of nitrous acid and nitrites with ammonia and with soil organic matter is needed to determine if appreciable gaseous losses of nitrogen occur through such reactions. The exact conditions under which they occur and to what extent should be determined. It is especially important that losses via ammonium nitrite be thoroughly explored. Experiments should be designed so that the chemical mechanisms of loss are separated from bacterial denitirification losses. Rarely has such a clear cut separation been reported in published data obtained under conditions that at least approximate practical conditions.

8. Nitrite added to or formed in an acid soil is chemically unstable and decomposes readily. What is the fate of this nitrogen? How much of the breakdown product, nitric oxide, escapes to the atmosphere and how much of it is adsorbed by the soil and oxidized chemically or biologically to nitrate? Data for soils of widely varying texture and reaction are required.

9. Quantitative data covering the various phases of nitrogen immobilization and nitrogen release by soil microorganisms are scarce and should be obtained for both cropped and uncropped soils. The available energy supply in the soil either in the form of added crop residues or as root excretions and decaying roots, is the controlling factor governing both rate and amount of immobilization and, likewise, rate and amount of release of nitrogen in an available form.

10. Good soil nitrogen balance data are scarce and much needed. Experiments should determine the recovery of nitrogen under various field conditions and, if feasible, explain what happens to the unrecovered nitrogen. Such unrecovered nitrogen commonly amounts to 10–20 percent or more of the total nitrogen added. The use of the N^{15} tracer technique is a "must" in experiments of this type.

PLANT NEMATOLOGY:

A Close Look At A Rapidly Developing Area of Biology

William F. Mai

NEMATODE parasites of animals were mentioned in early Egyptian records of 4500 B.C., but the existence of plant parasitic nematodes was unknown until the 17th century. This was not so unusual, because these nematodes vary from 1/3 mm. to 3 or 4 mm. long and are only the diameter of a human hair. It was not until almost 100 years after the discovery of the microscope that they were definitely recognized. Needham [1] recorded the discovery of the wheat gall nematode, *Anguina tritici,* in a letter to the President of the Royal Society of London on December 22, 1743. He published it in Philosophical Transactions the following year:

"Upon opening lately the small black Grains of smutty Wheat, which they here distinguish from blighted Corn, the latter affording nothing but a black Dust, into which the whole Substance of the Ear is converted; I percieved a soft white fibrous Substance, a small Portion of which I placed upon my Objectplate: It seemed to consist wholly of longitudinal Fibers bundled together; and you will be surprised, perhaps, that I should say, without the least Sign of Life or Motion. I dropped a Globule of Water upon it, in order to try if the Parts, when separated, might be viewed more conveniently; when to my great Surprise, these imaginary Fibres, as it were, instantly separated from each other, took Life, moved irregularly, not with a progressive, but twisting Motion; and continued so for the Space of Nine or Ten Hours, when I threw them away."

We now know that where such wheat galls are kept in a dry condition the nematode larvae may remain viable for more than 25 years. From a single gall up to 90,000 nematodes have been counted.

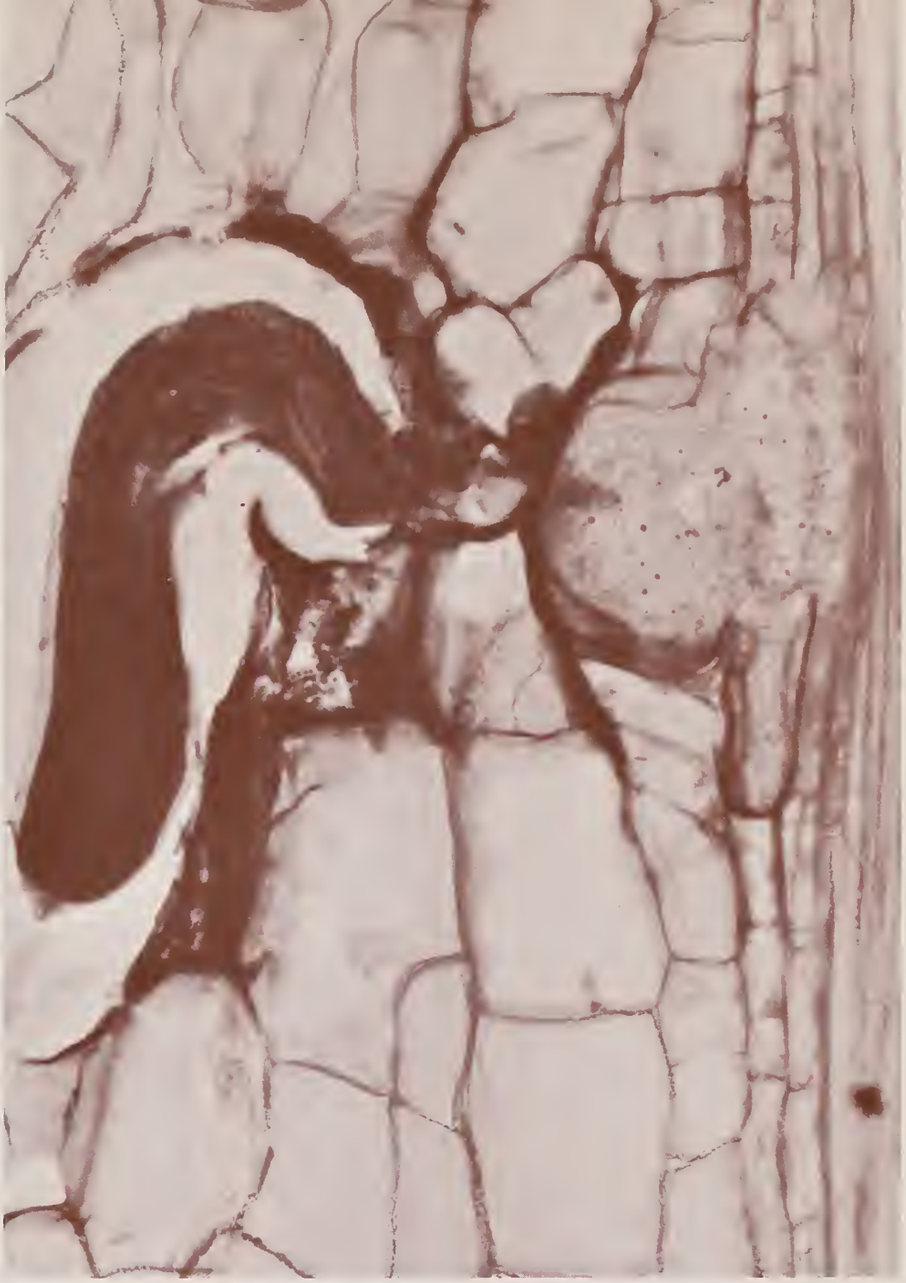
The nematodes that cause important diseases of man and other vertebrate animals have been studied extensively, but nematodes of invertebrate animals have received little attention.

It was not until 1855 that Berkeley [2] determined a root-knot nematode, *Meloidogyne* sp., to be the cause of galls on the roots of cucumber plants grown in a greenhouse in England. The first comprehensive paper on free-living nematodes was published by Bastion [3] in 1865. At the end of the 19th century a few plant pathologists were concerned with plant diseases caused by nematodes, but general recognition of the importance of nematodes as causal agents of plant diseases did not come until the middle of the current century. Because of their small size, lack of color, and life mostly underground, their presence was generally unobserved. Furthermore, symptoms of nematode damage are not usually obvious, because the most important damage occurs to roots and the above-ground symptoms are essentially the same as those caused by any factor that deprives the plant of an adequate and properly functioning root system. Thus it is rarely possible to recognize a nematode disease without root examination; determination of the causal species generally entails laboratory procedures.

[1] Needham, T. 1744. A letter concerning certain chalky tubulous concretions, called malm; with some microscopical observations on the farina of the red lily, and of worms discovered in smutty corn. Philos. Trans. Roy. Soc. 42: 634–641.

[2] Berkeley, M. J. 1855. Vibrio forming on excrescences on the roots of cucumber plants. Gardeners' Chronicle, April, 1855, p. 220.

[3] Bastion, H. C. 1865. Monograph on the Anguillulidae, or free nematoids, marine, land, or fresh water; with descriptions of 100 new species. Trans. Linn. Soc. 25: 73–178, 13 pls.



Until recently the techniques for isolation and identification of the nematodes from plants and soil were crude and limited.

Even by the 1930's, few people were concerned about the nematode problem. The scattered research projects at the State experiment stations were being conducted mostly by plant pathologists and plant breeders. Their interest centered on the diseases caused by plant-parasitic nematodes and on the development of resistant varieties of crop plants. The research program of the U.S. Department of Agriculture, although modest, was guided by pioneers such as Cobb, Steiner, Christie, and others. Some farsighted individuals, however, assessed the nematode situation with a great deal more seriousness than their contemporaries would admit. H. H. Hume, Dean of the School of Agriculture, University of Florida, said in 1938:

"If there were no nematodes in the South and they should suddenly appear in their present numbers, they would be seen as the pestilence they are. Funds for their control would be supplied quickly and in large amount. Such was the situation which allowed complete eradication of the Mediterranean fruit fly. But the nematode problem rouses no particular interest. Nematodes are always working havoc, taking their toll of crops, sometimes causing complete destruction. We blind ourselves by accepting them as a matter of course. The problem is here; we live with it; but that is no solution.

There must be a general awakening all along the line to the magnitude of this situation."

In 1943, interest in nematode diseases was stimulated by the discovery that the application of dichloropropene to field soils killed high percentages of nematodes. Here was the first practical chemical control for field use, a milestone in plant nematology. In addition the new nematocide provided a much needed tool for field research. Its use in a comparison of plant response in treated and untreated soils made possible estimates of nematode damage. During the past two decades, as research provided information, interest in nematodes and their intricate and important relationships to plants continued to grow.

HOST-PARASITE RELATIONSHIPS

PERHAPS one of the most complex areas of study in nematology is that of host-parasite relationships. We are not concerned solely with the mechanical, chemical, or physiological interference with the life of a plant. We must also be concerned with the changes these interferences bring about within the host through the direct and indirect introduction of disease organisms and the general decline that permits the invasion of other agents. Further complicating the study is the vast scope of the world of nematodes. More than 9,000 species have already been described; the unknown species probably far outnumber the known. Coupled with this fact is the increasing necessity for a more intensive type of agriculture that unquestionably helps the nematode attain a most favorable biological niche.

Most nematodes found below ground feed on small nonsuberized roots, although tubers, corms, and other storage organs may be attacked. Root feeding results in fewer roots as well as impaired uptake of water and minerals in the remaining diseased roots. Salivary secretions from feeding females of the endoparasitic [4] root knot nematodes cause the formation of large multinucleate cells so that infected roots become greatly swollen and distorted Although similar enlarged cells are formed when females of the cyst forming nematodes (*Heterodera* spp.) feed, the infected roots are only slightly swollen.

The endoparasitic lesion nematodes (*Pratylenchus* sp.) move throughout the cortex of the host root, feeding periodically and laying eggs, singly and in clumps. This results in death of scattered cortical cells, formation of lesions in the cortex, and often the destruction of nonsuberized roots. The feeding of one species of lesion nematode (*P. penetrans*) induces an enzymatic change in the cortical cells and produces a toxic compound which in turn destroys the cell. Nematodes of the closely related species (*Radopholus* spp.) have similar habits and biology.

Other effects of root feeding by nematodes—both endoparasites and ectoparasites—include excessive root branching, cessation of root elongation, retardation of root growth and elongation adjacent to feeding sites, and reduction in root growth without production of visible symptoms.

Many of the main forms of nematodes are sensitive to desiccation. Damage by those species feeding on above-ground plant parts is greatest, therefore, in greenhouses and other areas of high humidity. Feeding results in dead or devitalized buds, crinkled and distorted stems and foliage, seed and leaf galls, and leaf spots and necrosis.

Within the host-parasite complex, the fungus-nematode or the bacteria-nematode relationships are so great and so varied that they indicate a wide-open area for profitable research. For example, relatively weak fungal and bacterial root pathogens, once they gain entry into the root because of the presence of feeding nematodes, can cause tremendous damage. But in most instances we do not know if the presence of two or more pathogens on a single host results in an additive or symbiotic effect. Apparently, nematode attacks can sometimes lower the disease resistance of some plants, such as resistance to vascular wilt diseases caused by fungi and bacteria. Plants with nematode-damaged roots are more frequently susceptible to nutrient deficiencies and to cold and drought injuries. And to complicate the situation even more, we now have recent data indicating that fungal infection of roots may increase nematode buildup in these roots.

Although nematodes are known to transmit an ever increasing number of soil-borne virus infections, their importance in the damage caused by these virus diseases is difficult to ascertain. To accomplish a better understanding in the whole gamut of host-parasite-bacteria-fungal relationship,

[4] Plant parasitic nematodes may be divided into endoparasites and ectoparasites; the former generally enter and feed inside plant tissue while the latter generally feed on surface cells. Representatives of both groups occur on plant parts either above or below ground.

nematologists apparently need to know more about certain peripheral areas that until recently were of little concern. These areas include changes in plant constituents caused by parisitism, enzyme action, and the physiology, biochemistry, and genetics of nematodes.

NEMATODES AS AGRICULTURAL PESTS

NO accurate estimates are available as to the total impact of nematode damage to the world's food crops. But in the United States alone, the total annual loss from all crop diseases exceeds $2 billion—part of which can be attributed both directly and indirectly to nematode action. For example, in cotton alone, losses due to nematodes are estimated at more than $53 million annually. The cyst-forming sugar beet nematode has become a pest of major proportions in all established sugar beet growing areas of the United States.

Root knot nematodes with host ranges of several hundred crop plants do untold damage to greenhouse crops and to field crops grown in California and in the southeastern and southwestern agricultural areas of this country. Lesser, but extensive, damage occurs in other areas. Crops grown on lighter textured mineral soils and on organic soils tend to be the most severely damaged by nematodes. Perennial and annual crops grown without adequate rotation are often severely attacked. There are more important nematode diseases in warmer areas of the United States than in the cooler areas; however, some nematodes such as the golden nematode of potatoes, *Heterodera rostochiensis,* are more adapted to cold soils than warm ones.

Nematologists first became aware of the presence of the golden nematode in the United States after a Nassau County (N.Y.) potato grower appealed to the county agricultural agent for aid in determining why some of his potato plants were growing poorly in one of his fields. In 1941 the cause of his trouble was shown to be the golden nematode. Although surveys showed that approximately 18,000 acres on Long Island were infested, this nematode has not become established in any other part of the United States. In northern Europe and the British Isles, where the golden nematode is one of the most serious potato parasites, growers frequently harvest fewer potatoes than they plant.

Quarantine regulations based on research findings protect other potato-growing areas in the United States from the golden nematode. The most important benefit resulting from these regulations is that host crops—potatoes, tomatoes, and egg plants—are never grown on land known to be infested. To determine the extent of spread, yearly surveys—involving soil sampling and nematode extraction from these samples—are conducted on Long Island. Less frequent sampling is carried out in other potato-growing areas of the United States. Other important features of the golden nematode quarantines are: regulation of movement of soil, equipment, and plant materials on and from Long Island, and prohibition of seed potato production on Long Island.

Research on the golden nematode has brought a number of notable achievements: (1) effective survey methods, (2) an understanding of the life cycle of the nematode under Long Island conditions and the influence of environmental factors on population dynamics and survival, (3) a method for eliminating viable nematodes from equipment, (4) methods for achieving extremely high nematode kills in soil, and (5) highly resistant potato selections now being tested by commercial growers. The use of potato varieties with golden nematode resistance by Long Island growers will become an important part of the overall control program and thus provide additional protection to other potato-growing areas.

Nematodes will assume more importance within the next few decades as increased yields become of greater concern, and as soils in this country are used more intensively. Despite the widespread parasitism of every crop plant, less than 100 nematode diseases are now considered of serious proportions. Many other nematode attacks that cause less obvious or severe damage are generally unrecognized. Yield reductions and quality decreases are difficult to assess and ascribe to nematodes; however the use of effective nematode control measures will demonstrate that yields formerly considered "good" are not really good at all. For example, when nematodes and root infecting fungi and bacteria are controlled in strawberry plantings, yields have been several fold greater than those from untreated areas. A 10 to 30 percent yield reduction, such as attributable to many of the less destructive nematodes, is significant in the increasing worldwide competition for the crop dollar. Growers' problems with nema-

todes multiply as the best soils are cropped more intensively and as the nematode populations are permitted to increase in these soils. Nematodes are at least partially responsible for the growing number of orchard sites in which tree-fruit replants grow poorly. Citrus, cherries, peaches, apricots, apples, grapes and walnuts are all affected by replant problems.

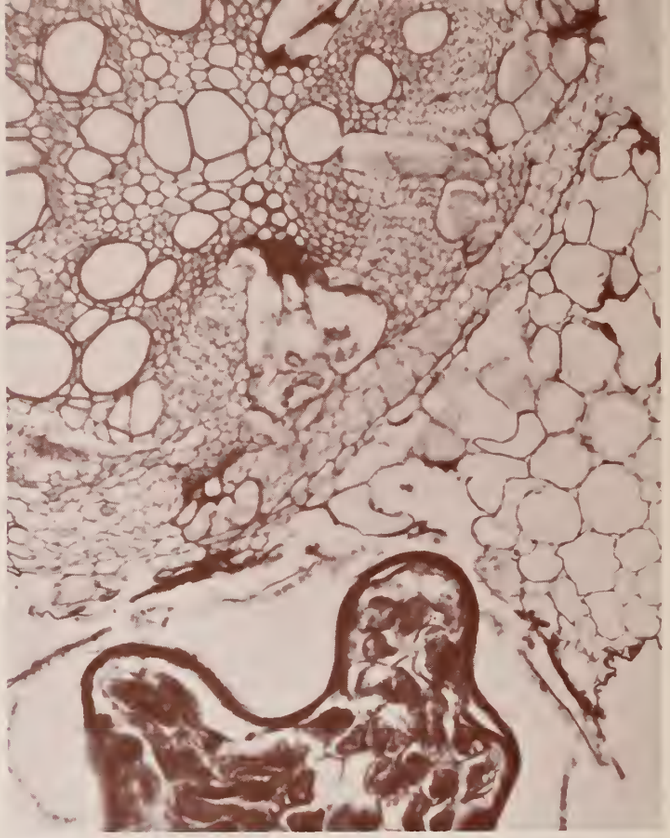
CONTROLLING NEMATODES

CROP rotation is the oldest and still most widely used field control measure for nematodes. Rotations, selected on the basis of yield alone without considering nematodes, often owe the resulting increased yields to the unwitting control of nematodes. In recent years, data from host range and population studies are utilized in planning rotations for nematode control in one or more of the major crops in the rotation.

The use of resistant varieties is one of the few practical ways to control nematodes attacking low acre-value crops. Breeding programs have developed commercial varieties of soybeans, peaches, and cotton with a high level of resistance to several species of the root-knot nematode. Likewise, breeding has resulted in varieties of alfalfa with a high resistance to the stem and bulb nematode, *Ditylenchus dipsaci*. In Europe, the British Isles, and in the United States, potato varieties have been bred that are highly resistant to the golden nematode and which have horticultural characteristics approaching those of commercial varieties. Recent data concerning the nature of plant resistance to nematodes indicate that usually a high percentage of nematodes either fail to mature or reproduce at a slow rate even though these nematodes enter resistant plants. A number of tolerant or resistant crop varieties have been developed by plant selection techniques.

Soil fumigation, although generally considered a modern technique, was used as early as 1884 when carbon disulfide was applied to over a million acres to control phylloxera of grape. This procedure undoubtedly also achieved nematode control. Carbon disulfide is not widely used today because of its high cost per acre and difficulty of application.

Dichloropropene provided the first practical chemical control for nematodes under field conditions. Upon injection into the soil prior to planting



a crop, this chemical changes from a liquid to a gas, slowly diffusing and permeating intercellular spaces and killing nematodes. It is relatively low in cost and easily applied to field soils without the use of a surface seal. A short time later the nematocidal properties of a similar chemical, ethylene dibromide, were demonstrated. Another breakthrough in chemical control came with the discovery that 1,2 dibromo 3 chloropropane could be used safely around the roots of some living plants. This chemical achieves a high kill of nematodes at lower concentrations than either dichloropropene or ethylene dibromide.

Water soluble nematocides, such as the dithiocarbamates, are effective only when applied with large quantities of water, because their movement is slow through soil when there is insufficient water to carry them. Highly volatile nematocides, such as methyl bromide and chloropicrin, are effective only when the treated soil is covered with a surface seal such as a plastic tarp. The recent use of tractor-drawn tarp-laying devices greatly increases the rate of chemical application.

Until recently, all the soil in a field was chemically treated. Now, when appropriate, only the rows or sites in which plants are to be grown are treated. This procedure, while killing fewer nematodes, is more economical than overall treatment.

Because of their greater effectiveness in warm rather than cold soils, nematocides are used widely in the warm areas of the country. This practice has led to the misconception that important nematode damage is confined to these warm areas. Large quantities of nematocides are used to control nematode diseases of the important cotton crop. The present cost of treatment with nematocide limits its use primarily to high acre value crops despite equal need on crops of lower value. A high percentage of soils planted to high acre value crops such as tobacco receive a preplant nematocide treatment.

Steam is widely used for nematode control in greenhouses; however, chemical control is equally satisfactory if suitable safety precautions are taken. Flooding, dry heat, electrocution, and trap cropping are generally considered impractical for nematode control. The use of certain chemicals in the soil to stimulate the hatching of nematodes from eggs, making them less resistant to unfavorable environmental factors, is also impractical.

TRAINING PLANT NEMATOLOGISTS

AFTER World War II interest in plant nematology increased rapidly, although progress was severely handicapped by the scarcity of persons trained in nematology—either teachers or research workers—and by the fact that few institutions offered more than a passing reference to nematodes in zoology and plant pathology courses.

But the vicious spread of nematode infestations and the attendant decline in quality of certain key crops pointed to an urgent need for action. The problems were clearly defined; the research manpower and know-how were inadequate. Thus was conceived the idea of conducting nematology workshops to help fill the void. Between 1950 and 1960, a series of these workshops held in various parts of the United States provided a marked stimulation of interest in all phases of plant nematology.

The workshop participants, generally drawn from a wide area covering several States, were primarily graduate students and young staff members with basic training in biology. The workshop teachers were outstanding scientists from universities and research stations located in various parts of the world. Major university-sponsored workshops varying from 1 to 6 weeks were held at the University of Maryland, the University of North Carolina (Raleigh), and Cornell University. For the workshops at the latter two universities, funds granted by the National Science Foundation paid for travel by students and teachers, and honoraria, living, and miscellaneous expenses for teachers. Two day workshops were held at such widely separated places as New York City, Orlando, Fla., Columbia, S.C., Yuma, Ariz., Toledo, Ohio, and San Juan, P.R. Although the students were primarily biologists employed by commercial companies, graduate students and university staff members also attended. The workshop teachers were outstanding. A considerable number of biologists received valuable training in nematology at these workshops; as a result the development of nematology in the United States was greatly accelerated.

Today the sustained interest in nematology has resulted in a marked increase in universities offering training in nematology both at undergraduate and graduate levels. University courses in nematology are generally included in entomology or plant pathology departments. One institution, the University of California, has a separate department of nematology.

WHAT THE FUTURE HOLDS

DESPITE the significant advances of the past decade, all fields of nematology still need extensive research. As late as 1959, our knowledge about nematodes and their control was described as being primitive.

Basic research on nematode physiology, biochemistry, and host-parasite relationships has gained impetus only comparatively recently. Although there has been some work in these areas with plant parasitic nematodes, most of it has been done on nematode parasites of man and animals. Because of the great differences in these two types of nematodes and in their environments, it is dangerous to assume that data obtained with the animal parasites apply equally to the plant parasites.

Sorely needed for more effective control of nematodes in the future is the development of versatile, economical, and easy to handle nematocides. For example, we need an improved nematocide that will give more effective results in the cold soils of northern growing regions. Nematocides that are nontoxic to a wide variety of plants would allow them to be applied at planting time and in the vi-

cinity of roots, thus resulting in better timing of applications and reductions in the amount of chemical needed. More rapid and effective methods of applying nematocides around roots, especially the roots of perennials, need to be developed. An "ideal" nematocide would be a systemic chemical which when sprayed on tops of plants would be translocated to the roots and kill nematodes feeding on these roots.

The fact that the nematode is now a pest of global concern, threatening world food supply, has prompted authorities in many countries to enact regulatory measures. Such action is particularly necessary where infestations are heavy and when plant materials are transported to other areas. Thus, arises the need for more effective nematocidal dips for nursery stock and other vegetative plant parts. The long distance shipment of plant parts is ever increasing. Often such plant material is grown in well-adapted areas where narrow crop rotation is practiced and there is frequent inadvertent buildup of nematode populations to high levels. Therefore, the danger of transporting nematode pests, on or around nursery stock, to new areas is high unless suitable treatments are applied. At present the only treatment generally recommended for killing nematodes inside plant tissue is hot water and this often results in plant damage.

The high cost of direct control and the persistence of nematodes in soil point to a need for greater emphasis on breeding resistant varieties. The development of more plant varieties with improved nematode resistance will result in untold benefits, especially to growers of tree fruits and forage crops. Before major progress in this field is possible, however, there must be a marked increase in numbers of research teams, consisting of both plant breeders and nematologists, actively engaged in the program. In addition, more effective testing methods for resistance to nematodes should be devised.

Fortunately, many colleges and universities recognize the wisdom of laying a better foundation in the training of nematologists who will ultimately carry on the work of breeding nematode-resistant varieties. Under this concept, therefore, the curriculum could include the whole gamut of agronomic sciences, genetics, entomology, soils, zoology, plant pathology, botany, plant physiology, as well as nematology itself.

Progress in basic research can be stepped up by adopting improved techniques of inoculation, culture and extraction of nematodes from soil and plant parts. Inability to grow plant parasitic nematodes on chemically-defined media under sterile conditions is a serious roadblock to many types of nematological research. Although several plant parasitic species can be grown on excised roots or callus tissue, the majority of species cannot even be grown in this manner. Large numbers of sterile nematodes of a single species are indispensable for studies on the physiology and biochemistry of nematodes and for studies on host-parasite relationships.

New approaches to nematode control will undoubtedly be found. Perhaps through research, a practical use can be made of natural enemies to control specific nematode diseases. Such enemies as fungi, bacteria, and invertebrate animals (including predaceous nematodes) greatly reduce nematode populations under certain naturally occuring conditions.

Ultimately, future nematological progress will depend on increased and more effective research activity in this new and important area of biology. The concerted support of basic programs by the Federal and State Governments and by the universities is urgently needed. Evidence of the benefits that accrue from the "team" approach may be found in the reports of regional research projects both in America and in Europe. From these projects have come new research techniques, new knowledge, clearer definitions of problems, the concept and realization of workshops to train research workers, cooperative development of manuals, taxonomic keys, and many other accomplishments.

With the recognition of the importance of nematology, man's control of nematode infestations on important food crops may very well become one of the most dramatic developments of the twentieth century in the protection of the world's food supply.

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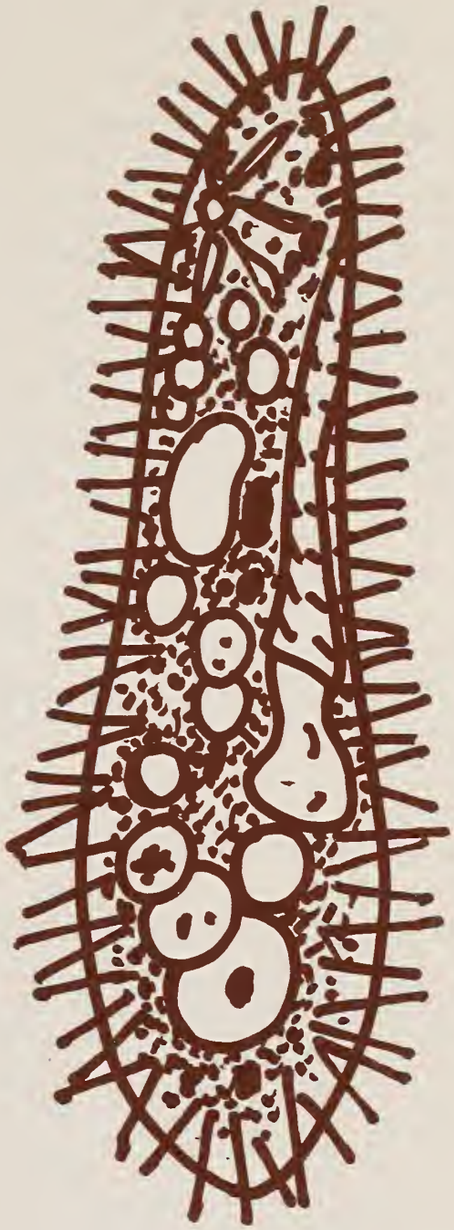

Physiology of THE RUMEN PROTOZOA

Jose Gutierrez

RUMINAL protozoa are currently providing some insight into the mechanisms operating in the nutrition of ruminant animals. The main areas of research cover their cultivation, metabolism, and predation of associated bacteria. Protozoa can be placed in two major groups: the holotrichs which have rows of cilia over the entire cell as in paramecium, and the oligotrichs with tufts of cilia or membranelles at the anterior part of the body. The holotrichs include the genera *Isotricha* and *Dasytricha* which readily digest soluble carbohydrates such as glucose and sucrose but do not attack cellulose (Oxford, *7*).[1] The oligotrichs include the genera *Entodinium, Diplodinium* and *Ophryoscolex*. The latter three species digest starch, protein and bacteria. Other functions of the protozoa are the production of volatile fatty acids that are absorbed through the ruminal wall and serve as an energy source for the ruminant. They metabolize higher fatty acids such as oleic and stearic, and hydrogenate unsaturated fatty acids (Williams et al. *9*). The protozoa appear to have most of the substrate enzymes which the bacteria possess, and are able to compete with the bacteria in their ability to metabolize carbohydrates, fats, and proteins. It is of considerable importance to acquire knowledge of the metabolic capabilities of the bacteria and protozoa in order to have a better

[1] Italic numbers in parentheses refer to "Literature Cited" p. 34.

understanding of their contribution to the nutrition of the host.

PROTOZOAL POPULATIONS

THE anaerobic ruminal ciliates are found only in the rumen of grazing animals. Establishment of the ciliates in the rumen occurs several weeks after birth and the various species differ in the time required for them to become established. Small species of *Entodinium* are the first to populate the rumen, followed by *Isotricha, Diplodinium* and *Ophryoscolex*. The numbers vary from animal to animal and are affected to some extent by the diet being fed. Counts will oftentimes range between 1,000–50,000 protozoa per ml. and occasionally higher. When animals are on a hay or pasture diet, the holotrichs *Isotricha* and *Dasytricha* are generally found in larger numbers than in animals on a grain diet. Starchy rations favor the genera *Entodinium* and *Ophryoscolex*.

BACTERIAL INGESTION

PREDATION of associated bacteria has been demonstrated for several species of ruminal ciliates and the bacterial feeding is specific for certain types of bacterial strains. With *Isotricha prostoma,* starvation of the protozoan cells for 72–96 hours decreased the reserve amylopectin granules and allowed microscopic observation of the bacterial feeding. *I. prostoma* selected and ingested only certain rods from among many types of ruminal bacteria. To identify the bacteria ingested by the protozoa, starved protozoa were allowed to feed on the mixed ruminal bacteria, then were washed and the contents quickly cultured for bacteria.

Several strains of bacteria have been isolated in pure culture. Three of the rod strains isolated were rapidly ingested by *I. prostoma* when fed to the ciliates. The feeding reaction appears to be an agglutination phenomenon in which the bacteria adhere to the posterior part of the cell around the mouth area, and are gradually propelled inward by the ciliary action of the gullet. Bacterial feeding has also been demonstrated in the oligotrich protozoan species *Entodinium* and *Diplodinium*. In the latter two species, the bacteria which were ingested by a direct feeding mechanism were similar to *Streptococcus bovis*. Predation of the bacteria by the protozoa affects the overall population of the bacteria in the rumen as has been shown when bacterial counts are made on animals faunated with protozoa and compared with animals without ciliate populations.

CARBOHYDRATE METABOLISM

RECENT studies have expanded our knowledge of carbohydrate breakdown by the rumen protozoa. Ruminal ciliates are involved in the metabolism of starch, cellulose, and soluble carbohydrates such as sucrose, glucose and fructose, while *Entodinium* and *Ophryoscolex* readily metabolize starch grains. In the holotrich protozoa the reserve amylopectin granules within the cell allow an endogenous carbohydrate metabolism that furnishes the host with a steady supply of organic acids. Calculations based on average protozoan populations and on the amount of acid produced per protozoan cell show that protozoa furnish approximately one-fifth of the host's energy requirements. Amylase enzymes have been isolated from the ciliate *Epidinium ecaudatum,* and from cell-free extracts of *Entodinium ecaudatum* (Abou Akkada and Howard, *1*). In the latter species starch appears to be the sole carbohydrate that is utilized. Products of carbohydrate fermentation were butyric and acetic acids and small amounts of propionic and formic acid. Digestion of cellulose by the protozoan *Diplodinium* has been demonstrated by using cellular extracts (Hungate, *6*). The cellulase was found to be active at a pH between 4.0 and 6.6. Pectic materials constitute a significant portion of the plant matter ingested by ruminants, and the breakdown of pectin has been shown to occur using suspensions of *Ophryoscolex, Isotricha* and *Dasytricha*. Galacturonic acid was the chief product of hydrolysis of pectic substances by the holotrich species. Cell free extracts of *Isotricha* and *Dasytricha* can hydrolyse polysaccharides such as raffinose and inulin (Howard, *5*).

CULTIVATION EXPERIMENTS

NUMEROUS early experiments have been made at culturing the ruminal ciliates. The successful culture of *Eudiplodinium neglectum* in a medium containing grass, cellulose and inorganic salts allowed growth for 22 months (Hungate, *6*). The small oligotrich protozoan, *Entodinium ecaudatum*

was maintained in culture for 18 months on a medium of rice starch, dried grass and rumen fluid (Coleman, *2*) while the large oligotrich *Epidinium ecaudatum* has been cultured for six months (Gutierrez and Davis, *3*). More recently, Quinn et al. (*8*) reported on the continuous culture of *Entodinium, Diplodinium,* and *Isotricha.* They described a complete medium which contained amino acids, vitamins, nucleic acids, carbohydrates, and

fatty acids for the ruminal ciliates. Cultivation of the protozoa was possible when factors such as anaerobiosis, pH, feed rate, oxidation-reduction potential and salinity were controlled.

Some of the protozoal growth requirements have been followed in *in vitro* culture work. *Isotricha intestinalis* has been grown for short periods (three weeks) in a medium that included ruminal fluid, inorganic salts, ground alfalfa, and wheat plus viable bacteria. When the bacteria were eliminated from the cuture either by autoclaving or by the use of antibiotics, the protozoan culture would gradually diminish in numbers and finally die out. Improved results in the longevity of the cultures have been obtained with the protozoan *Epidinium ecaudatum,* which has been cultured for six months with known bacteria, starch, and ground alfalfa.

Growth studies with *Epidinium* by using strains of bacteria isolated from the protozoa have provided some preliminary information. Protozoa washed free of external bacteria and debris were used to start cultures that were provided with strains of the lactic acid producing *Streptococcus bovis* originally isolated from *Epidinium.* Usually 80–100 epidinia were inoculated into 5 ml. of the culture medium which contained 0.02 percent each of ground rice starch and alfalfa. The 5 ml. culture flasks supported peak populations of 5–6000 protozoa. Cultures were maintained in the laboratory for six months and had to be transferred every 24 hours, but would show higher populations if transferred and given fresh substrate twice daily. The starch and alfalfa must be kept at a low level to limit the growth of the bacteria. Attempts to grow the protozoa free of bacteria were not successful.

NITROGEN METABOLISM

PROTOZOA are a significant source of protein for the ruminant as they are digested and absorbed from the alimentary tract. Data on the total nitrogen per protozoan cell allow an estimation of the nitrogenous contribution of the protozoa to the host under average conditions. This amount has been found to be approximately 33 grams of protein supplied to the host each day. Amino acid analysis by paper chromatography of *Ophryoscolex* hydrolysates has shown serine, glycine threonine, alanine, tyrosine, methionine, arginine, proline, valine, phenylalanine, leucine, isoleucine, cysteine, aspartic acid, glutamic acid, and lysine. The amino acids are absorbed through the digestive tract after lysis of the protozoan cells. Feeding trials with rats showed protozoan protein had more nutritive value than either bacterial or yeast protein. Ammonia was found to be the nitrogenous product of casein metabolism in the protozoan *Ophryoscolex.*

LIPID METABOLISM

THE biochemical alteration of lipids in the rumen by microorganisms has been of some interest since the depot fats are mainly the saturated type. Ingested feedstuffs contain both unsaturated and saturated classes of lipids. The use of washed suspensions of the ruminal ciliates, *Isotricha prostoma* and *Entodinium* showed that C^{14} labelled oleic, palmitic, stearic, and linoleic acids are concentrated within the cells during short incubation periods (Gutierrez et al. *4*). Radioautographs demonstrated that oleic-1–C^{14} was hydrogenated to stearic

acid by *I. prostoma,* and warburg manometric data showed the sodium salts of oleic, valeric, caproic and acetic acids stimulated gas production by *I. prostoma.* Analysis for lipid content of *Isotricha intestinalis* cells gave a value of 9.1 percent on a dry weight basis, while for *Entodinium simplex* the amount of lipid was 6.3 percent and for bacterial cells the lipid fraction was 6.8 percent (Williams et al. *9*). Fractionation of the lipid material on silicic acid columns gave mostly phospholipid along with sterol esters, mono-, di-, and triglycerides. On a cellular dry weight basis the protozoa showed more fermentative activity against fatty acids such as oleic acid than did the associated rumen bacteria. Experiments with C^{14} labelled higher fatty acids indicated the protozoa contained stearic, palmitic, and traces of oleic, linoleic, and lauric acids. These protozoal long chain fatty acids are made available to the ruminant as the cells are lysed and absorbed through the digestive tract. Ruminal protozoa, therefore, may be assumed to contribute to the saturation of lipids which are components of forages and feedstuffs, and thus modify the fat supply which is available to the host.

FUTURE PERSPECTIVES

SEVERAL aspects of the metabolism of the rumen protozoa hold promising prospects for future research. The maintenance of animals inoculated with pure cultures of the protozoa offers an opportunity for collecting large suspensions of single species of protozoa easily prepared for biochemical and physiological studies. Washed suspensions of the different species of ciliates may be used to obtain further information on their lipid and nucleic acid metabolism. Little work has been done on factors that influence the variability of the protozoal populations in ruminants except for some preliminary work on the relationship between frequency of feeding and protozoal numbers.

Lack of information also exists on the complexity of nutritional interactions between the rumen protozoa and bacteria, although preliminary contributions have been made on some aspects of bacterial feeding by the protozoa. Vitamin requirements of the protozoa is another area which has received little attention in the past, and may provide useful information on unidentified growth factors supplied to the protozoa by the associated bacteria. The metabolism of pesticide chemicals which are ingested with the feedstuffs and liable to enzymatic degradation by the protozoa is yet another prospective problem that is beginning to yield useful knowledge. These partially unexplored areas of research hold promise of useful contributions to the field, and could logically serve as starting points for further investigations.

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FORUM

ANY critical appraisal of an animal science research program raises a philosophical question which must eventually be answered in some manner. That question is: "Where will animal research be done in the future?" From the viewpoint of the animal husbandman or animal scientist this question should result in much soul searching.

Animal science in itself is not a science but a group of sciences. Tools from each science are employed to study the basic phenomena of animal life. Animal scientists must be competent in one or more of the areas of chemistry, zoology, microbiology, mathematics, statistics, physics, physiology or other basic disciplines.

Animal husbandmen or animal scientists many times are frustrated farmers or ranchers. They have ridden "high in the saddle" through the years, primarily because the animals with which they have worked are surrounded by romance and intense public interest. This was adequate two or three decades ago, but today the "halo" surrounding the conventional animal husbandman is dimming commercially and academically. The following examples illustrate the point.

Showing of fancy poultry is fast becoming an activity of the past, and collegiate poultry judging teams have only limited interest among students and staff. The reason is that commercial poultry production today is built upon performance rather than on many of the fine points once associated with production. Judging activity in the agronomic field has been dropped at many institutions. Judging activities in the livestock and meats area continue at most institutions despite academic criticism. It will, however, probably continue to be an important part of the teaching program in animal husbandry departments as long as millions of head of livestock change hands each year on the basis of value judgment of two or more people.

There are some who are very critical of judging activity, but there are also many who feel that until a substitute for judging becomes a reality, the criticism is perhaps unwarranted. It should be pointed out, however, that livestock judging is assuming a new look—judgment based upon actual commercial value, not color or the other many fine points once considered so important. Animal scientists must redouble efforts to find new methods to obtain objective measurements of our meat animals so that value can be determined more accurately than by visual appraisal.

The animal husbandman is very sensitive to public opinion because, no doubt, of the close association with the producer and his practical problems. The animal husbandman consequently has been satisfied to do the applied research and let someone else do the basic work. There are many who feel that the animal scientist must devote more time, effort, and resources to the basic and fundamental problems of animal life. Young men are being trained today to go in this direction. The disturbing fact is that too few animal science departments have the laboratories and equipment to attract the top young people to that department. Too few animal scientists have worked hard to secure facilities for basic research. Too many administrators have elected to provide laboratories and facilities for basic research to departments other than animal science. Unfortunately, animal science departments many times have not had the interest in the basic laboratory concept or have been unable to convince the university administration of the need for basic facilities and staff competence to use such facilities.

Where will the animal research be done in the future? Consider briefly livestock management. Many young animal scientists consider management research as too plebeian, a nonscientific area of endeavor. Today, however, many facts that have been known to animal scientists because of their close association with animals are being rediscovered by the animal behaviorist or animal psychologist, couched in new scientific language and published in reputable scientific journals. The animal scientist hadn't considered this type of work sufficiently important to publish for the use of the entire scientific fraternity.

Basic nutrition research is being done today by departments for biochemistry, colleges of veterinary

medicine, or biological departments possessing adequate laboratories and tools, in addition to the few departments of animal science that have basic tools and facilities. Research in physiology is concentrated in departments of physiology, zoology, and microbiology and in colleges of veterinary medicine or medicine. The need for basic studies in physiology in the animal science area has never been greater. Adequate facilities for fundamental physiological research are seldom found in animal science departments. Genetic research is done in departments of zoology, microbiology, botany, agronomy, and other plant departments as well as in departments of animal science.

One answer to the question of where will the research be done is: "Where it is being done today." Many feel that expansion of basic research in the animal field will be in departments or colleges other than departments of animal science. The principal reason is that university administrators frequently favor an expansion of existing facilities in departments other than animal science rather than duplication of such facilities in an animal science department. It would appear that the animal scientist should stand up and be counted, work hard to acquire facilities to do basic and fundamental research, and concentrate upon training young people to work upon basic animal problems. The alternative is to continue to be a conventional animal husbandry or animal science department—unchanged from that of 20 to 30 years ago—and depend upon scientists of other disciplines to do the basic and fundamental animal work.

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* * *

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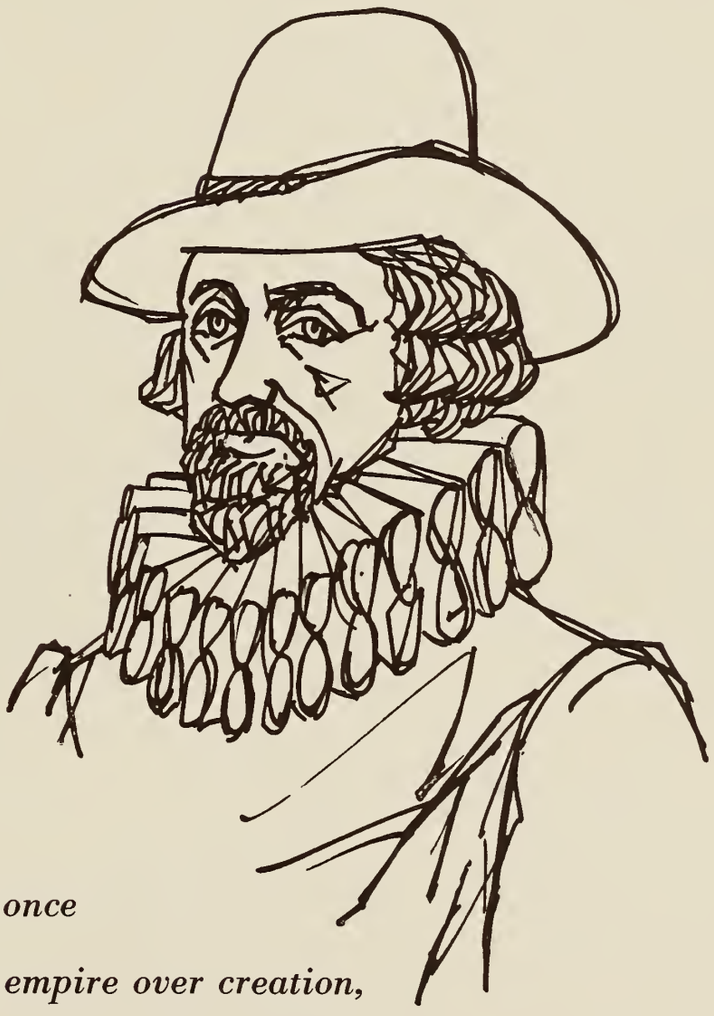

"For man, by the fall, lost at once
his state of innocence and his empire over creation,
both of which can be partially recovered even in this life—
the first by religion and faith,
the second by the arts and sciences."

FRANCIS BACON 1561–1626